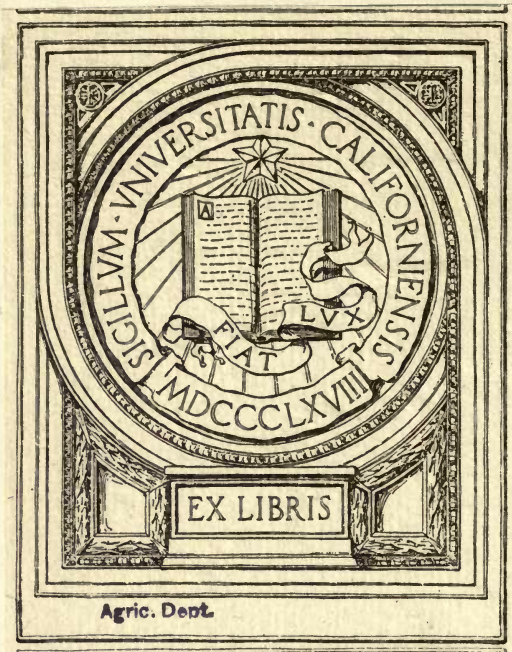


MANUAL

OF

HISTOLOGY AND BACTERIOLOGY

INCLUDING

A CONCISE STATEMENT OF THE IMPORTANT FACTS OF MICROSCOPIC TECHNIQUE AND URINALYSIS,

AND

A LABORATORY COURSE OF SEVENTY PRACTICAL EXERCISES

WITH

PROVISION FOR NOTES AND DRAWINGS.

BY

WILLIAM OSBURN, A. M.

Professor of Histology, Bacteriology, and Botany,
Meharry Medical College.

1899,
PRESS OF THE MARSHALL & BRUCE COMPANY,
NASHVILLE, TENN.

CONTENTS.

PART 4.—URINALYSIS.

PREFACE.

This modest manual has been prepared by the writer for his classes in Histology and Bacteriology. The condensed statement of many important facts of these subjects will be appreciated by those who have a limited amount of time for their study. The absence of diagrams may be a disappointment to some and, at first thought, appear as a disadvantage; but the study of nature is primarily the study of objects, and not of books and diagrams, though these are admitted helps. Its end should be the interpretation of facts and the demonstration of truth, and this is accomplished by going direct to nature, the source of facts and the embodiment of truth. A fact is any reality, and truth is the correspondence between a proposition and a reality—a quality rather than an essence, the substantive suggested by the adjective *true*. Not every proposition is true, but every reality in nature embodies a truth. When we prove the agreement between a proposition and the real thing at issue we demonstrate the truth. The same thing is accomplished, and doubtless a higher discipline attained, when we accurately observe a reality of nature and then frame a proposition that truthfully expresses that reality. One of the highest acts of the human mind is correctly to observe a fact and accurately state the truth embodied in that fact. A *diagram* may embody the truth. When a student correctly interprets nature and then prepares a diagram that truthfully represents that interpretation, he has performed an act of the highest disciplinary and practical value. Nothing will convey to the mind of the teacher a student's conception of a subject so fully as an effort on the part of that student to represent by a diagram that which he observes. It is well, therefore, to encourage students to copy nature rather than the conceptions of others, even though their delineations may at times appear crude. The motto of the teacher should be: "Nothing goes in this laboratory without drawings."

The provision made for drawings in this manual will greatly facilitate that work. The foot-notes will indicate what the student is to observe and illustrate, and by lettering the structures drawn, as indicated in the descriptive foot-note, time and labor will be saved. In connection with this work, black-board diagrams, hand-made charts, and lantern projections can be used, that the student may have the clearest possible conception of what the microscope reveals before beginning his task with pencil or pen.

A word to the student will not be amiss. The faithful student will not be content to let another do his work. He will be determined to make every demonstration count. He will strive to be accurate in his observations and painstaking in his notes and drawings. Blots, finger marks and erasures will be studiously avoided. In the laboratory he will keep everything in its place and clean up after each exercise. He will not be satisfied with the statements of one author, but will seek information from every available source. These seem minor things, but they enter into character. Like straws, they show the direction of the current.

The writer desires to acknowledge his gratitude to kind friends for encouragement, and especially to Dr. G. W. Hubbard, Dean of Meharry Medical College, and J. H. Holman, M.D., Instructor in Histology and Bacteriology. He is under obligation to many sources for the materials herein presented, but more especially to the valuable texts of Stirling and Piersol on Histology, and of McFarland and Williams on Bacteriology. The student who would investigate these subjects more thoroughly is advised to secure one or more of these works. The writer is aware that no new facts are herein presented, and is fully conscious of the imperfections and shortcomings of this manual, but hopes that the plan proposed will be of service to some, and with this hope sends it forth to accomplish its purpose among others of kindred nature.

WILLIAM OSBURN.

NASHVILLE, TENN., August, 1899.

INTRODUCTION.

The scope of this manual is intended to cover a brief statement of the important facts of Microscopy, Histology, Bacteriology, and Urinalysis.

Microscopy, in a liberal sense, is the microscopic study of natural objects. In a more restricted sense, it is the study of the microscope, its structure and manipulation, and of microscopic technique.

Histology is that department of Biology which treats of the minute anatomy of plants and animals. *Animal Histology* deals with the microscopic structure of animals; *Vegetable Histology,* with that of plants. Animal Histology consists of two departments—namely, *Normal Histology* and *Pathology.* Normal Histology deals with cells and tissues in their normal, or healthy, state. Pathology deals with these structures as affected by disease. There is an intimate relation between Normal Histology and Pathology. Disease affects the cells, and the student must understand the character of the cells in a condition of health before he can adequately comprehend their pathological condition.

Bacteriology is that department of Botany which treats of bacteria—minute, unicellular, chlorophylless fission-plants.

Urinalysis is the examination of the urine by physical, chemical, and microscopical tests, by means of which its normal or pathological condition is determined.

PART I.

MICROSCOPY.

CHAPTER I.

THE MICROSCOPE AND ACCESSORIES.

The *microscope* is a lens or combination of lenses designed to aid the eye in the examination of minute objects.

KINDS OF MICROSCOPES.

There are two kinds of microscopes, simple and compound. A simple microscope is a single lens (or group of lenses) which is so used that the object to be examined is between the focus and the lens. It produces an erect virtual image. A compound microscope is a combination of lenses, by means of which an inverted image is produced, and this is viewed by the eye, not the real object.

STRUCTURE OF THE COMPOUND MICROSCOPE.

The following parts should be carefully studied, and the use of each part thoroughly understood:

The *base* rests upon the table and supports all the other parts.

The *pillar* is the upright column which supports the arm.

The *arm* is attached to and works upon the pillar by means of the hinge-joint.

The *reflector* is the mirror by which the object to be examined is illuminated.

The *stage* is the platform upon which rests the slide containing the preparation to be studied. Clips are springs attached to the stage to hold the slide in position.

The *aperture* is the circular opening in the stage.

The *diaphragm* is the circular disk which regulates the amount of light required for illumination.

The *body* is the cylindrical attachment supported by the arm.

The *draw-tube* is the tube which moves within the body.

The *nose-piece* is attached to the lower end of the body and bears the objectives.

The *objective* is the lens attached to the body or nose-piece. It often consists of several pieces of glass cemented together.

The objectives commonly used are the two-thirds, one-sixth, and one-twelfth inch. By a two-thirds inch objective is meant one whose magnifying power is equal to that of a lens whose focal length is two-thirds of an inch.

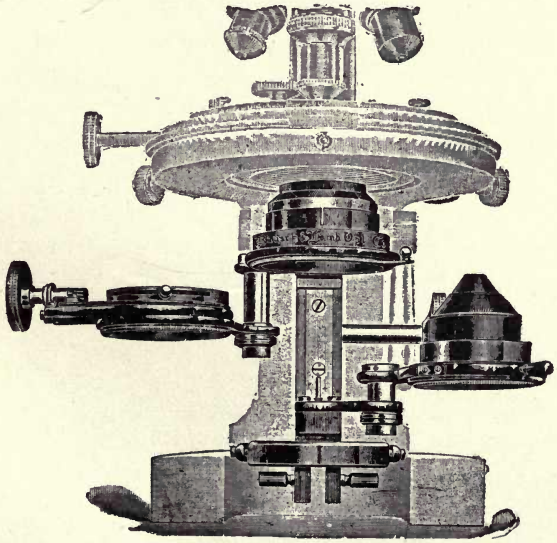
The *eye-piece* comprises the lenses which fit into the upper end of the draw-tube. It contains a *field-glass* and *eye-glass,* each a plano convex lens, with the convex surface downward.

The *coarse-adjustment* consists of the two vertical milled-heads and the rack and pinion.

The *fine-adjustment* is secured by the horizontal milled-head.

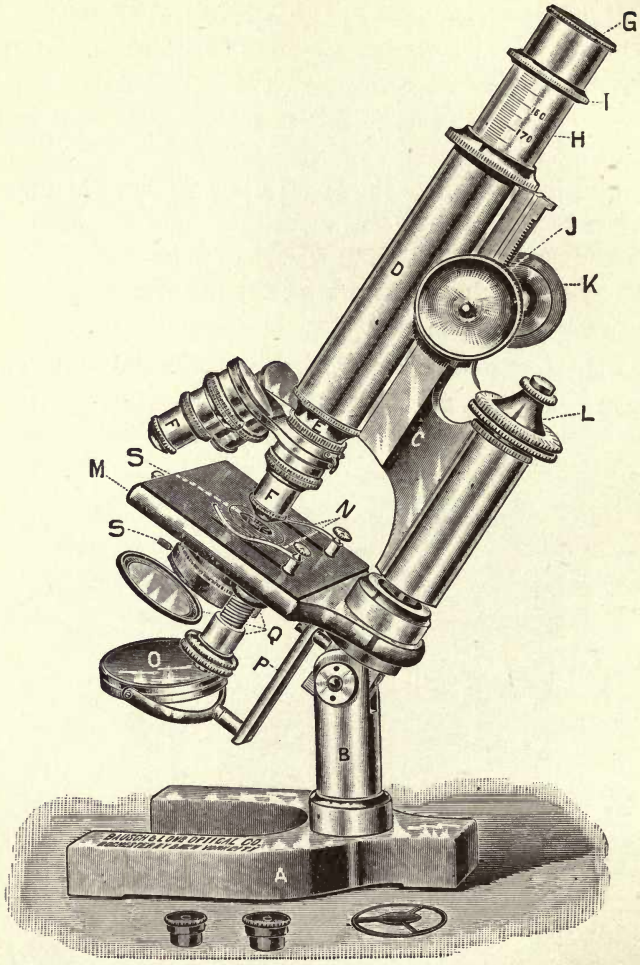
Laboratory exercise No. 1.—Examine a compound microscope and make out each part named above. Make a study of the diagram on page 10.

Substage Attachment with Condenser.



Bausch & Lomb Optical Co., Rochester, N. Y.

MICROSCOPE, SHOWING PARTS.

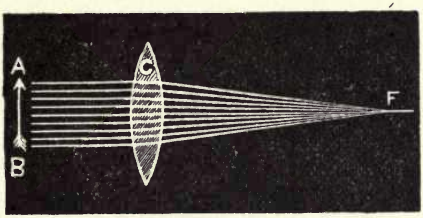


Bausch & Lomb Optical Co., Rochester, N. Y.

Parts: **A**, base; **B**, pillar; **C**. arm; **D**, body; **E**, nose-piece; **F**, ojectives; **G**, eye-piece; **H**, draw-tube; **I**, collar; **J**, coarse-adjustment; **K**, milled-heads; **L**, fine-adjustment; **M**, stage; **N**, clips; **O**, mirror; **P**, mirror-bar; **Q**, substage; **R**, substage bar; **S**, diaphragm.

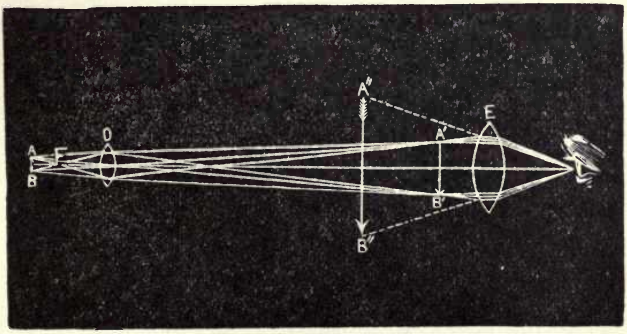
PHYSICAL PRINCIPLES.

The image produced by a compound microscope is a magnified inverted real image. Imagine an innumerable number of lines proceeding from the object to be examined in a direction perpendicular to the long axis of the objective. These lines represent the rays of light proceeding from all points of the object. As they pass through the objective they are bent, or refracted, and converged to a common point called the principal focus. No image is produced at this focus. The accompanying diagram illustrates how this focus is produced.



A B, object; C, lens; F, principal focus.

It is at the conjugate foci that the image is formed. There are as many conjugate foci as there are emergent points on the surface of the object. They are situated between the principal focus and the eye-piece. Let us suppose an innumerable number of emergent rays of light to have proceeded from any point of the object. These rays, striking the lens of the objective upon every point of its surface, are refracted and converged to a point called the conjugate focus. The following diagram will illustrate how the



A B, object; O, objective; A′ and B′, conjugate foci; E, eye-piece; A′ B′, inverted image produced by the objective; A″ B″ magnified inverted image viewed by the eye.

image is formed at the conjugate focus and how this image is magnified by the eye-piece, thus producing the magnified, inverted, real image which is viewed by the eye of the observer.

MANIPULATION AND CARE OF A MICROSCOPE.

Handling.

The microscope should be handled with more than ordinary care. In moving it from place to place it should be caught firmly by the pillar. Reagents of any kind should not be allowed to come in contact with it. Alcohol will destroy the lacquer, and acids will produce corrosion of its surface. The hands, therefore, should be kept perfectly clean.

Cleansing.

The microscope may be cleaned with a linen cloth. Reagents should not be used in cleansing the objectives and eye-piece without the direction of the teacher. To remove balsam from the objective, alcohol or xylol may be used, but should be quickly removed with Japanese paper, a soft paper especially used for cleaning lenses.

Obtaining a Focus.

To obtain a focus, place the slide upon the stage so that the object to be examined appears in the center of the aperture; adjust the reflector so as to illuminate the object. Lower the objective by means of the coarse-adjustment until it is below the focal point; then, with the eye at the eye-glass, work upward until the object appears, making the focusing perfect by means of the fine-adjustment.

Cautions.

To save harm to the eyes in the use of the microscope it is a good plan to keep both eyes open; let the eye be used as though viewing some distant object; there should be no conscious strain to obtain a focus, but let the hand with fine-adjustment aid the eye; do not use the microscope long at a time, so as to produce an aching sensation in the eye.

Allow nothing to touch the lenses, except Japanese paper or soft linen, which may be used in cleaning them.

ACCESSORIES.

The sub-stage.—This is an attachment supported beneath the stage and is designed to receive the iris diaphragm, Abbe condenser, etc.

Iris diaphragm.—This is supported by the sub-stage and is so constructed that the aperture for admitting light may be regulated by turning a milled-head.

Abbe condenser.—This is an apparatus containing a lens of very short focus, and is capable of producing intense illumination. It is supported by the sub-stage.

Mechanical stage.—This is attached to the microscope so as to work above the stage, and is designed to hold the slide and so change its position as to bring every part of the object into the field of view.

Camera Lucida.—This is an apparatus which is attached to the tube of the microscope, and is designed to assist in making diagrams of the object studied.

Polariscope.—Polarization consits in reducing vibrations of light to one plane. This is accomplished by the polariscope, which consists of a polarizer and an analyzer. The polarizer consists of a crystal of Iceland spar, which by double refraction separates the ordinary from the extraordinary ray. The analyzer generally used is a Nicol's prism, which consists of a crystal of Iceland spar split diagonally and the pieces then cemented together with Canada balsam. The Canada balsam produces the total reflection of the ordinary ray, while the extraordinary ray passes through. The analyzer is used to detect polarized light.

Micrometer.—There are two kinds ofmicrometers—the stage micrometer and the eye-piece micrometer. The stage micrometer is a small glass slide, upon which is a graduated scale, the graduations being in millimeters and tenths of a millimeter. It is used in determining the magnifying power of the microscope.

Laboratory exercise No. 2.—***To obtain a focus.*** **Place upon the stage a prepared slide; adjust the reflector so as to illuminate the object; using first the low power, by means of the coarse-adjustment lower the objective until it is below the focal point. If the objective be a two-thirds inch, the focal point will be somewhat less than two-thirds of an inch from the object. Now, with the eye at the eye-piece work upward until the object appears in view. Perfect the focusing by using**

the fine-adjustment. When using the microscope it is always a good plan to keep the hand upon the fine-adjustment, using it constantly to bring different planes of the object into the field of vision.

To determine the magnifying power. Place upon the stage a stage-micrometer and focus with both eyes open. The lines upon the micrometer will also be seen by the eye not in use. Place under the microscope or upon the stage a sheet of white paper. Now, with a pencil mark the apparent, or magnified, distance between two lines. Knowing the real distance, one-tenth of a millimeter, the magnification can readily be determined. For example, should the magnified distance between two lines be thirty millimeters, the real distance being one-tenth of a millimeter, the magnification would therefore be ten times thirty, or 300.

Microtome.—The microtome is an apparatus employed in cutting microscopic sections of tissues. It is provided with a microtome knife, a knife-carrier, and the milled-head which operates a mechanism for regulating the thickness of the sections. The student microtome manufactured by the Bausch & Lomb Optical Company, of Rochester, N. Y., is a most excellent instrument for all ordinary work.

Paraffin Bath.—This is designed for use in infiltrating and embedding tissues in paraffin. The heat should be so regulated as to keep the paraffin as near the melting point as possible. Where gas is available this may be accomplished by means of a thermostat.

Cornet Forceps.—This is a forceps especially useful in holding cover-glasses when staining preparations of sputum, bacteria, etc.

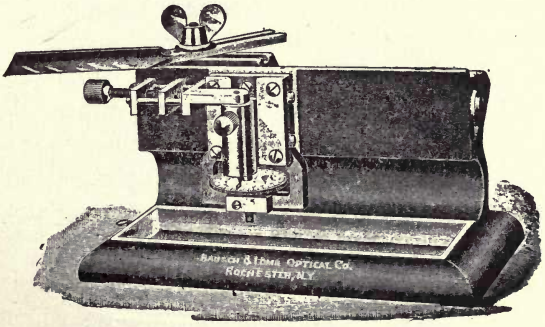
Centrifuge.—This apparatus utilizes the centrifugal tendency and is employed to separate substances of different specific gravity. It is provided with two important attachments, the sedimentation tubes and carrier and the hæmatokrit. The sedimentation tube contains fifteen cubic centimeters and is graduated into 100 equal parts, up to ten cubic centimeters, and above that each cubic centimeter is graduated into four equal parts for the measurement of reagents employed. By means of these the solid matter in urine, water, etc. may be precipitated and the exact per cent determined.

The hæmatokrit is provided with graduated tubes; each tube is fifty millimeters in length and is divided into 100 equal parts. The diameter of the bore is 0.5 millimeter. These tubes are used in determining the percentage composition of the blood. By revolving the handle of the centrifuge seventy-seven times in a minute the hæma-

tokrit is caused to rotate 5,000 times. This rapid rotation precipitates to the outer end of the tube the red blood corpuscles, which are of the highest specific gravity. Next to these will be arranged the white corpuscles, which are heavier than the plasma, while the plasma fills the remaining portion of the tube. A tube is also provided for sputum, by means of which bacteria may be precipitated, thus increasing the accuracy of a microscopic analysis.

Slide and Cover-glass.—The slide consists of a piece of glass one inch wide and three inches long, and is used to receive the object to be examined. The cover-glass is a thin piece of glass, rectangular or circular in shape. In cleaning slides and cover-glasses, tissue paper or a linen towel may be used. They should always be seized by their edges, never allowing the fingers to touch the flat surfaces.

Student Microtome.



Bausch & Lomb Optical Co., Rochester, N. Y.

NOTE.—Nearly all the illustrations of this manual are from electrotypes kindly loaned the author by the Bausch & Lomb Optical Company, of Rochester, N. Y. Students and others desiring to purchase microscopes and microscopic supplies will find this firm courteous in its dealings. The goods sent out by this house are reasonable in price and first-class in quality.

CHAPTER II.

MICROSCOPIC TECHNIQUE.

I. THE HISTORY OF THE SLIDE.

The history of a slide from the crude tissue to the finished mount includes the following processes: Fixing, hardening, infiltrating, embedding, sectioning, fixation, staining, dehydrating, clearing, mounting, labeling.

1. Fixing.—This process consists in so killing the cells as to preserve them in their natural form and structure. The reagents commonly used for this purpose are alcohol, corrosive sublimate, chromic acid, Perenyi's fluid, etc. To fix tissues in absolute alcohol, they should be allowed to remain from one to six hours, according to the character of the specimen. Objects are left in Perenyi's fluid from three to twelve hours and then transferred to seventy per cent alcohol. Specimens hardened in corrosive sublimate solution should be removed in one to three hours, according to size. To fix tissue in chromic acid requires from a few days to a few weeks. After fixing in the last reagent, the tissue should be thoroughly washed with water and then run through increasing strengths of alcohol in the dark. Picro-sulphuric Acid, Erlicki's Fluid, Muller's Fluid, and Flemming's Solution are also commonly used for this purpose.

2. Hardening.—This consists in dehydrating the tissues so as to make them rigid and suited for sectioning with the microtome. For this purpose alcohol is commonly used. The tissue should be run through increasing strengths—seventy per cent, eighty per cent, ninety per cent, ninety-five per cent, and absolute alcohol. Objects should not be allowed to remain long in absolute alcohol (from one to five hours), as this renders the tissue brittle and causes it to crumble under the knife of the microtome. The objects may remain in the other strengths of alcohol about twenty-four hours for each.

3. Infiltrating.—This process consists in removing the hardening agent and filling up the pores of the tissue with an embedding me-

dium. As soon as the object is removed from absolute alcohol it should be thoroughly dried with blotting paper. If it is desired to embed with paraffin, the specimen should be placed in xylol, chloroform, turpentine, or cedar oil for at least twelve hours, and then transferred to a solution of paraffin and xylol, allowing it to remain twelve to twenty-four hours. It may now be transferred to melted paraffin, which should be kept as little above the melting point as possible. After remaining until it becomes thoroughly saturated with the paraffin, which usually requires from twelve to twenty-four hours, it may then be embedded. To infiltrate with celloidin, two solutions are necessary, a thin and a thick. The celloidin is dissolved in equal parts of absolute alcohol and ether. To make the thin solution, use five grams of celloidin in 100 cc. of the mixture. The second solution should have the consistency of thick sirup. The dehydrated tissue is placed in a mixture of equal parts of absolute alcohol and ether from twelve to forty-eight hours, then in the thin solution for about the same period. It may remain in the thick solution twenty-four hours, or longer if desired. It is then ready for embedding.

4. Embedding in paraffin—Embedding L's or paper boxes may be used. To accomplish this process, the following method may be pursued: Place upon a pane of glass a clean piece of paper. Arrange the embedding L's so as to form a receptacle of the required size. Pour into this a small quantity of melted paraffin. Now arrange the tissue in the position desired and fill the receptacle with paraffin. As soon as the paraffin becomes sufficiently hardened the embedding L's may be set in a vessel containing ice-cold water. Care should be taken that the water does not run over the top of the paraffin.

To Embed with Celloidin.—A cork or block of wood which has been soaked in equal parts of absolute alcohol and ether may be used. Place upon the cork or block a small quantity of thick celloidin. Then place in position the piece of tissue and cover it with the celloidin, adding a little at a time, layer after layer as each hardens, until the tissue is completely embedded. The whole may now be transferred to chloroform for one or two hours, and then to seventy-five per cent alcohol, where it may be left indefinitely.

5. Sectioning.—This consists in cutting thin sections of the object to be examined. It is accomplished by the use of a razor, or microtome knife, which may be used free-hand or with a microtome. To cut paraffin sections, first pare the block to the right size and then fasten it in the clamp of the microtome. The paraffin should be kept at the right temperature. This may be accomplished by using cold or hot water, applying it with a camel's-hair brush, or with the heat of the hand or a flame. The knife should be drawn at a slight angle. If sections curl, they may be placed upon the surface of moderately warm water to flatten them. Curling may be prevented by holding the sections in place with a camel's-hair brush as they are cut.

Celloidin sections are cut in the same way, with the exception that the knife and celloidin block must be kept constantly flooded with seventy-five per cent alcohol. The sections when cut may be removed to a vessel containing seventy-five per cent alcohol, and there kept indefinitely.

6. Fixation.—This consists in attaching sections to the slide. Paraffin sections may be affixed with collodion mixture, or with egg-albumen and glycerine. To affix with collodion, make a thin layer with camel's-hair brush upon the slide. Then apply the section, flattening it out with finger or brush; now apply the heat of a spirit or Bunsen flame until the paraffin melts, being careful to avoid excessive heating, such as would injure the tissue. To affix with egg-albumen and glycerine, a thin coating is made with a camel's-hair brush, and the section is then transferred to the center of the slide, care being taken to flatten it out with finger or brush. Heat is then applied to coagulate the albumen. To avoid overheating, the slide should be frequently applied to the surface of the hand. When the paraffin becomes thoroughly melted the section is usually properly affixed.

If desired to affix celloidin sections, a drop of ether may be applied to each section after placing it in the desired position, or they may be affixed with the collodion and clove-oil mixture in the following manner: Apply a thin layer of collodion mixture to center of slide; when the collodion is dry apply the section, together with the thin piece of paper upon which it has been placed, and press upon

the whole with a dry blotting paper. The section will now adhere and the paper may be removed. Now cover the section with a thin layer of collodion mixture, and it will be thoroughly affixed.

Centering.—The process of fixation should also include the centering of the section. This may readily be accomplished by means of a diagram of the slide, which may be drawn upon the under surface of the cover of a mailing box. See laboratory exercise No. 3.

7. Staining.—This process consists in tinting the structures of the section with certain stains, so as to produce a differentiation of the different elements and render them more readily studied. It depends upon the principle that certain structures have an affinity for certain stains, but not for others. For example, a stain that will affect the protoplasm and nucleus may have no effect upon the cell-wall. A stain that will affect certain cells may have no effect upon others. Hæmatoxylin will stain the leucocytes, but not the red corpuscles. Eosin will stain the red corpuscles, but not the white. Staining should always be preceded by certain processes so as to prepare the tissue to receive it. First, if a paraffin section is to be stained, the paraffin must be removed. This is accomplished by immersing it in xylol, turpentine, chloroform, or benzole. These reagents dissolve out the paraffin. The xylol, etc., may then be removed by applying alcohol. Should an aqueous solution of any stain be used, the section should be washed with water before applying the stain, and followed with the same. If the stain be alcoholic, its application should be preceded and followed by alcohol of the same strength. The different staining methods are given on page 23.

8. Dehydrating.—This consists in the removal of water from the specimen, water being generally the enemy of the histologist. It is accomplished by running the section through increasing strengths of alcohol, using first an alcohol of the same strength as the staining solution. The object of this is to prevent the precipitation of the stain, by which the preparation becomes filled with dark granular masses.

9. Clearing.—This consists in the removal of the alcohol so as to prepare the sections for balsam and in so clearing up the section as to render it transparent. By closely observing the change in color

or by placing the finger nail beneath the section, the student can determine whether the process is complete. The reagents commonly used for clearing purposes are creosote, cedar oil, xylol, benzole, clove oil, and aniline oil.

10. Mounting consists in permanently attaching the cover-glass for the protection of the specimen. Balsam and glycerine-jelly are generally used for this purpose. For laboratory work the balsam method will be found the most convenient. Place upon the cover-glass, while holding it between the fingers, a drop of balsam, and then (balsam down) let it fall gently upon the section. After centering the cover-glass, apply gentle pressure by means of a dissecting needle, so as to force the balsam out to the edges of the glass. Should too much balsam be used, it may be removed (when thoroughly dried) with a pen-knife. This method will be found more satisfactory than to undertake its removal with cloth or brush by means of xylol. The safest plan is to use just enough balsam, no more, no less.

11. Labeling.—Two labels should be used, one on each end of the slide, and they should be so applied that the edges of the labels will be the same distance from the edges of the slide. The left hand label should indicate the number of the preparation, the staining fluid, the mounting medium, the date, and the name of the student. The right hand label should indicate the kind of tissue, the character of the section (whether transverse, longitudinal, vertical, or oblique), the condition of the specimen (whether normal or abnormal), and the animal from which it has been obtained. After labeling, the preparation should be placed in the slide-box in a horizontal position, with the cover-glass up, and kept in that position until the balsam hardens.

II. SPECIAL TREATMENT OF TISSUES AND ORGANS.

The structures required for microscopic work may be obtained from some animal, such as the cat, rabbit, or guinea pig. Should a cat be used, it may be killed by placing it under an inverted bowl (resting upon a heavy sheet of paper upon a table or floor), and then inserting a sponge saturated with chloroform. In twenty minutes the animal will be dead and ready for injection.

Injecting.—To inject the animal, the following process may be

pursued: Sever the costal cartilages on each side of the sternum; lift up the sternum and bend it forward so as to expose the heart. Make a slit into the right auricle to allow the escape of the blood. Snip off the end of the heart and slit open the left ventricle. Insert the canula of the injecting syringe into the aorta, carefully tying the same upon the end of the canula. Now, having filled the syringe with normal saline solution at body temperature and having filled the canula with the same, using pipette, attach the two, and with a gentle pressure force the liquid through the system until the arteries and veins have been thoroughly relieved of blood. Repeat the same process, using Carter's Carmine Mass. By observing the lips and other structures it can be determined when the circulatory system is filled with the injecting fluid. Now, make a ligature around the aorta, just beyond the canula, and the syringe can be removed. In fifteen minutes the tissues can be cut up into small blocks (these blocks should be in the form of cubes or rectangles from 1 cc. to 2 cc. in size), and placed in the fixing fluids.

Epithelium.—For purposes of study epithelium may be obtained from the casts of a frog or newt, from the scrapings of the human lip, from the throat of a frog, and the scrapings of the trachea of a pig. This material may be readily obtained by macerating the object in weak alcohol. Keep the specimens in eighty per cent alcohol, and use when required.

Cartilage.—Fix in absolute alcohol; harden with increasing strengths of alcohol, and embed in paraffin. Stain with carmine.

Mucous Tissue.—Fix small pieces of the umbilical cord with absolute alcohol, harden with alcohol, embed in celloidin, and stain with hæmatoxylin.

Bones and Teeth.—Fix in ninety-five per cent alcohol three days; decalcify in a saturated aqueous solution of picric acid or in a ten per cent solution of nitric acid. This process will require from five to ten days. When the bone or tooth is thoroughly softened, transfer to ninety-five per cent alcohol, changing in three days to fresh alcohol. Embed in celloidin. Specimens embedded in paraffin should not be overheated.

Muscle.—Fix in absolute alcohol; harden with increasing strengths of alcohol; embed in paraffin; stain with lithium carmine.

The muscle of a salamander will be found excellent for demonstrating the structure of the fibers.

Brain and Spinal Cord.—Fix and harden in Muller's fluid or Erlicki's fluid, two weeks for the former and fifteen days for the latter. Wash thoroughly in water before embedding, which may be done in paraffin.

Heart.—Fix in absolute alcohol; harden with alcohols; embed in paraffin; stain with hæmatoxylin.

Blood Vessels.—Fix and harden with alcohol; embed in paraffin or celloidin; and stain with lithium carmine or hæmatoxylin and eosin.

Lymphatic Glands.—Fix and harden with alcohol; embed in celloidin; and stain with hæmatoxylin and eosin.

Skin.—Fix and harden with alcohol; embed in paraffin; and stain with hæmatoxylin and eosin.

The Spleen.—Fix and harden with alcohol; embed in paraffin; stain with hæmatoxylin and eosin.

Œsophagus.—Fix with corrosive sublimate or Perenyi's fluid; harden with alcohol; embed in paraffin; and stain with hæmatoxylin and eosin.

Stomach.—Fix with corrosive sublimate; harden with alcohol; embed with paraffin or celloidin; stain with hæmatoxylin.

Intestine.—Fix with corrosive sublimate; harden with alcohol; embed in celloidin; stain with hæmatoxylin and eosin.

Tongue.—Fix with absolute alcohol; harden with alcohols; embed in paraffin; and stain with hæmatoxylin and eosin.

Trachea.—After filling the trachea and lungs with a two-tenths per cent solution of chromic acid, suspend them in a large volume of the same for two days; then cut into small pieces and place in two-tenths per cent of chromic acid for two or three weeks; wash thoroughly with water; embed in celloidin; and stain with hæmatoxylin.

Lungs.—These may be treated as the trachea; embed in paraffin or celloidin; and stain with lithium carmine or hæmatoxylin and eosin.

Liver.—Fix with corrosive sublimate, one per cent solution, twenty-four hours; harden with alcohol; embed in paraffin; and stain with hæmatoxylin and eosin.

Kidney.—Fix and harden with alcohol; embed with paraffin; and stain with hæmatoxylin and eosin.

Ovary.—Fix and harden with alcohol; embed in paraffin; and stain with hæmatoxylin and eosin. The Fallopian tube may be treated in the same way.

Uterus.—Fix and harden with alcohol; embed in paraffin; and stain with hæmatoxylin and eosin.

Testis.—Fix with corrosive sublimate, and harden with alcohol; embed in paraffin; and stain with lithium carmine.

Eye.—Cut across the eye so as to partly divide it into an anterior and posterior half; suspend in 150 cc. of chromic acid (0.25 per cent) for twenty-four hours; sever the halves and renew the fluid; after several days' wash in water, and harden in alcohol in the dark; embed in paraffin; and stain with hæmatoxylin and eosin.

Pancreas.—Fix with Flemming's solution, twenty-four hours; harden with alcohol; stain with lithium carmine; embedding may be done in paraffin.

III. STAINING METHODS.

Sections to be stained may be fresh specimens or those that have been cut from embedded tissue. They may be free—that is, unattached to the slide, or affixed. The following schemes for staining are intended to be sufficiently comprehensive to include all of these conditions:

No. 1. METHOD FOR STAINING FRESH VEGETABLE SECTIONS.

(1) Apply section to slide and add rosanilin violet, one to five minutes.

(2) Wash in water to remove excess of stain.

(3) Dry with blotting paper and add glycerine to dehydrate.

(4) Remove excess of glycerine and add glycerine again to thoroughly dehydrate.

(5) Wipe off excess of glycerine and add xylol twice.

(6) Apply to cover-glass a drop or two of xylol-balsam, and, having wiped off the excess of xylol from the slide, drop it gently (balsam down) upon the section. Then apply gentle pressure with dissecting needle to spread out the balsam.

(7) Label and keep in a horizontal position until the balsam is hardened.

Vegetable Sections.—To stain paraffin or celloidin sections of plant structures, the methods are practically the same as those given below for animal objects.

Methods for Animal Sections.

No. 2. CARMINE METHOD FOR FREE SECTIONS.

(1) Apply section to slide and wash with thirty-five per cent alcohol.

(2) Add lithium carmine sufficient to cover section, one to five minutes.

(3) If necessary, remove excess of stain with acid alcohol, five to ten seconds.

(4) Dehydrate with increasing strengths of alcohol—thirty-five per cent, seventy-five per cent, ninety-five per cent, and absolute.

(5) Wipe off excess of alcohol, and when section is partly dry add creosote to clear up, five to ten minutes.

(6) Wipe off excess of creosote and mount with balsam.

(7) Center cover-glass and apply pressure to spread out the balsam.

(8) Label and lay aside in horizontal position, cover-glass up, until the balsam hardens.

No. 3. CARMINE METHOD WITH AFFIXED PARAFFIN SECTIONS.

(1) Apply to the center of the slide a thin layer of collodion-clove-oil mixture.

(2) Center and attach the section, applying the heat of a spirit or Bunsen flame.

(3) Immerse in xylol two minutes and in turpentine ten minutes to remove paraffin. Sections immersed in turpentine alone should remain twenty minutes.

(4) Wash with alcohol, decreasing strengths, using thirty-five per cent alcohol last.

(5) Apply lithium carmine, one to ten minutes.
(6) Remove excess of stain with acid alcohol.
(7) Dehydrate with alcohol, increasing strengths.
(8) Dry and clear up with creosote, five to ten minutes.
(9) Wipe off excess of creosote and mount in balsam.
(10) Center cover-glass.
(11) Label and lay aside in horizontal position until balsam hardens.

No. 4. CARMINE METHOD FOR AFFIXED CELLOIDIN SECTIONS.

(1) Center section and affix with collodion mixture.
(2) Stain with lithium carmine, one to five minutes.
(3) Remove excess of stain with acid alcohol, five to ten seconds.
(4) Apply seventy per cent alcohol.
(5) Apply eighty per cent alcohol.
(6) Apply ninety-five per cent alcohol a few seconds.
(7) Clear up with creosote.
(8) Remove excess of creosote with blotting paper.
(9) Mount with balsam and center cover-glass.
(10) Label and lay aside in a horizontal position.

No. 5. HÆMATOXYLIN METHOD FOR FREE SECTIONS.

(1) With section on slide, apply alcohol of the same strength as staining solution.
(2) Stain with diluted hæmatoxylin, one to ten minutes.
(3) Remove excess of stain with thirty-five per cent alcohol.
(4) Dehydrate with alcohols, increasing strengths.
(5) Clear up with creosote or cedar oil.
(6) Center section and apply balsam and cover-glass.
(7) Center cover-glass.
(8) Label and lay aside in horizontal position until balsam hardens.

No. 6. HÆMATOXYLIN METHOD FOR AFFIXED PARAFFIN SECTIONS.

(1) Apply to slide a thin layer of egg-albumen and glycerine.

(2) Center section and flatten it by gently touching with end of finger.

(3) Apply heat of flame until paraffin melts (sections that have been flattened upon water should be heated much longer than others).

(4) Remove paraffin with xylol or turpentine.

(5) Remove xylol, etc., with alcohol, decreasing strengths.

(6) Stain with diluted hæmatoxylin, one to ten minutes.

(7) Remove excess of stain with thirty-five per cent alcohol.

(8) Dehydrate with alcohol.

(9) Clear up with creosote, five to ten minutes.

(10) Wipe off excess of creosote and mount with balsam.

(11) Center cover-glass.

(12) Label and keep in horizontal position until balsam hardens.

No. 7. HÆMATOXYLIN METHOD FOR AFFIXED CELLOIDIN SECTIONS.

(1) Center section and affix with collodion mixture.

(2) Stain with diluted hæmatoxylin, one to ten minutes.

(3) Remove excess of stain with acid alcohol.

(4) Apply seventy per cent alcohol.

(5) Apply eighty per cent alcohol.

(6) Apply ninety-five per cent alcohol a few seconds.

(7) Clear up with creosote, five to ten minutes.

(8) Remove excess of creosote with blotting paper.

(9) Mount with balsam and center cover-glass.

(10) Label and lay aside in a horizontal position until balsam is hardened.

No. 8. HÆMATOXYLIN-EOSIN METHOD.

(1) If desired, affix section to slide with egg-albumen and glycerine or collodion and clove-oil.

(2) If a paraffin section, remove paraffin with xylol or turpentine or both; remove xylol, etc., with alcohol.

(3) Apply thirty-five per cent alcohol.

(4) Stain with diluted hæmatoxylin, one to five minutes.

(5) Apply thirty-five per cent alcohol to remove excess of stain.

(6) Stain with alcoholic eosin about five minutes.

(7) Apply ninety-five per cent alcohol to remove excess of stain and dehydrate.

(8) Clear up with creosote.

(9) Remove excess of creosote and mount with balsam.

(10) Center cover-glass and label.

(11) Lay aside in horizontal position until balsam hardens.

Staining Unicellular Organisms.

It is often desirable to examine material without staining. This is accomplished by placing upon the glass slip a drop of the material to be examined and applying cover-glass. A hair placed under the cover-glass will prevent the object from being crushed and allow of free motion in the case of living organisms. Should it be desired to stain such preparations, two methods may be pursued, irrigation and cover-glass staining.

No. 9. IRRIGATION AND STAINING MICRO-ORGANISMS.

(1) Place upon the slide a drop of material to be studied.

(2) Apply cover-glass.

(3) At the edge of the cover-glass, by means of a pipette, place a drop or two of the reagent or stain.

(4) By means of a triangular piece of blotting paper applied at the opposite edge of the cover-glass, absorb the moisture from the preparation, thus drawing under the stain.

No. 10. COVER-GLASS PREPARATIONS.

(1) Make a thin spread of the substance to be examined upon a sterilized cover-glass.

(2) Using a Cornet forceps, dry the preparation by holding it between the fingers above a flame.

(3) When dry pass the cover-glass three times through a flame, keeping the preparation up.

(4) Apply stain.

(5) Wash in distilled water by dipping the cover-glass in the water two or three times.

(6) Examine as a water mount or, if desired, dry and mount in balsam.

(7) Label and lay aside in a horizontal position until balsam hardens.

Note.—The above method may be used for all simple staining. Special methods, however, are often used, and they will be given as required.

Laboratory Exercise No. 3.—*Centering and labeling.* Upon the under side of your box-cover make an outline of a slide. The pencil should have a needle point. Connect opposite angles and place over the intersection of the lines a cover-glass. Be sure that the center of the cover-glass coincides with the center of the diagram. Now, carefully make an outline of the cover-glass. This outline may be used for centering both the sections and the cover-glass. Make a drawing of this outline on page 29, also a drawing of a slide with labels and cover-glass *in situ.* Fill in the forms of labels in second diagram, using the following data: A transverse section of the muscle of a normal cat was stained with lithium carmine and mounted in balsam on October 1, 1891, by John Smith.

Drawings. For this work the student should provide himself with a No. 5 or a No. 6 H Faber pencil, a small rule or triangle and a sheet of thin blotting paper. The pencil should be kept sharpened to a needle point. The majority of students will say: I cannot draw. An honest and faithful effort will often produce gratifying results. Let every line mean something. Be scrupulously neat in all your work. Remember that this work will furnish a better exhibit of character and ability than any other task of the laboratory.

Abbreviations. The following abbreviations are employed in this text:

Transverse section—T. S.
Longitudinal section—L. S.
Vertical section—V. S.
Low power—L. P.
High power—H. P.
Cubic centimeter—c. c.
Micro-millimeter—μ.
Millimeter—mm.
Gram—g.

MICROSCOPIC TECHNIQUE.

Diagram of Slide and Cover Glass.

Diagram for centering: (a) Slide; (b) Cover Glass; (c) Center.

Labels *in situ*.

Labeling: (a) Label properly filled out for reagents, etc.; (b) Label descriptive of section.

CHAPTER III.

REAGENTS AND STAINS.

In preparing the following reagents it is well to remember that the weight of a cubic centimeter of water is one gram, and that a liter contains 1,000 cubic centimeters. The formulæ that are given are those most commonly used and are briefly stated:

NORMAL FLUIDS.

Distilled water.—A supply of distilled water should be constantly at hand for the preparation of the reagents and stains.

Normal saline.—This is prepared by dissolving one part, by weight, of sodium chloride in 150 parts of distilled water.

MACERATING FLUIDS,

Dilute alcohol.—This may be prepared by mixing one part of ninety-five per cent alcohol with two parts of distilled water. Other fluids used for this purpose are solutions of potassium bi-chromate, two per cent, and caustic potash, twenty-five per cent.

DECALCIFYING FLUIDS.

Picric acid.—Make a saturated aqueous solution of picric acid. This is an excellent fluid for decalcifying bones, serving at the same time as a staining reagent. Crystals should be added from time to time, so that some undissolved crystals will always remain in the bottom of the vessel.

Nitric acid.—Use a ten per cent volumetric solution in water. Decalcification occurs in five to ten days.

FIXING REAGENTS.

Absolute alcohol.—Specimens should remain in this reagent from one to six hours, according to size.

Perenyi's fluid—

Nitric acid (ten per cent).....40 cc.
Chromic acid (0.5 per cent).....30 cc.
Alcohol .....30 cc.

A good reagent for embryos and adult tissues. Time, three to twelve hours; dehydrate with alcohol.

Erlicki's fluid—

Potassium bi-chromate	2.5 grams.
Cupric sulphate	0.5 grams.
Water	100 cc.

A good reagent for general use, for embryos and nervous tissues. Time, ten to fifteen days; dehydrate with alcohol.

Corrosive sublimate—

Aqueous Solution—

Corrosive sublimate	1 gram.
Water	95 cc.

Alcoholic Solution—

Corrosive sublimate	1 gram.
Alcohol (ninety-five per cent)	99 cc.

Used for general purposes, and specially for alimentary tract. Time, twenty-four hours, hardening in alcohol, to which a few crystals of iodine have been added.

Chromic acid. —Use a 0.5 per cent solution, dehydrating with alcohol in the dark.

Muller's fluid—

Potassium bi-chromate	25 grams.
Sodium sulphate	10 grams.
Water	1,000 cc.

Pulverize the solids before adding water, and use a piece of camphor in the solution to prevent the formation of fungi. Good for general use and especially valuable for central nervous system. Requires from two to six weeks. Wash in water for several days, and dehydrate with alcohol.

Flemming's fluid—

Chromic acid (one per cent solution)	46 cc.
Osmic acid (two per cent solution)	12 cc.
Glacial acetic acid	3 cc.

Especially valuable for delicate tissue. Time, two to twenty-four hours; dehydrate with alcohol.

HARDENING REAGENTS.

Muller's fluid, *corrosive sublimate solution*, *chromic acid*, and others of the reagents named above may be used for hardening purposes. For general use, *alcohol* will be found invaluable. The tissue should be passed through increasing strengths of alcohol, seventy per cent, eighty per cent, ninety per cent, ninety-five per cent, and absolute. It should be allowed to remain twenty-four hours in each, except that one to six hours will suffice for absolute alcohol. Ethyl alcohol should be used, or, in lieu of this, methyl alcohol makes a good substitute. To prepare absolute alcohol, dehydrated copper sulphate may be added to the ethyl or methyl alcohol. This will absorb the water present.

EMBEDDING MEDIA.

Paraffin and celloidin are extensively used for embedding tissue. The process for each has been fully explained in the chapter on "Microscopic Technique."

FIXATIVES.

Collodion and clove oil mixture.—Mix one part of collodion with three parts of clove-oil.

Egg-albumen and glycerine.—Filter the whites of several eggs and add to the filtrate an equal volume of glycerine. To the mixture add a few drops of carbolic acid or a small piece of thymol to prevent putrefaction.

PARAFFIN SOLVENTS.

Xylol, turpentine, chloroform, and benzole are commonly used to remove paraffin from sections. A good plan is to immerse the slide containing the section for a few moments in xylol, and then transfer to turpentine for ten minutes.

STAINING SOLUTIONS.

The following staining preparations are those most frequently used, and will be found adequate to the work required by this text. Should others be needed, the formulæ can be obtained from more advanced works.

Hanstein's rosanilin violet—

Methyl violet..... .... 1 gram.
Fuchsin..... .... 1 gram.
Distilled water .....100 cc.

Note.—This is a valuable stain for vegetable sections. It should be diluted for use as desired.

Lithium carmine—

Carmine... .... 2.5 grams.
Lithium carbonate (saturated solution) ....100 cc.

The carmine should be dissolved in cold solution. Sections stain rapidly, and should be decolorized with acid alcohol.

Dalafield's hæmatoxylin—

1. Hæmatoxylin .... 1 gram.
2. Absolute alcohol ..... 6 cc.
3. Ammonia alum (saturated sol.)...100 cc.
4. Glycerine .... 25 cc.
5. Methyl alcohol ..... 25 cc.

Process.—Dissolve (1) in (2); add this solution to (3); expose to air and light for a week; filter and add (4) and (5); allow it to stand for a long time exposed to air and light.

Eosin—

Alcoholic Eosin for Sections.—Make a saturated alcoholic solution. This is used as a ground stain in connection with hæmatoxylin; also as a blood stain.

Magenta for blood, etc.—

Magenta .... 1 gram.
Alcohol (eighty-five per cent).....50 cc.
Water ....150 cc.
Glycerine ....200 cc.

Methylene blue for blood—

Make a saturated aqueous solution.

Carter's carmine mass for injecting—

Carmine	3 grams.
Strong ammonia	6 cc.
Glacial acetic acid	6 cc.
Coignet's French gelatin	7 grams.
Water	80 cc.

Process.—"Place the finely cut gelatin in 50 cc. of water for five hours; dissolve the carmine in a mortar with a little water, and add the ammonia; let stand for two hours and then pour into a bottle, rinsing the mortar with the remainder of water; place the gelatin and water on a water-bath until the gelatin melts. To the carmine fluid add the acetic acid, a few drops at a time (rinsing mortar thoroughly) until the fluid becomes crimson. To the melted gelatin add the crimson carmine, little by little, with continual stirring. Keep in a cool place with surface covered with methylated spirit. When wanted for use, dissolve on water-bath and filter through flannel wrung out of hot water." (Fearnley's Method.)

CLEARING AGENTS.

Those commonly used are cedar oil, creosote, clove-oil, xylol, and aniline oil. Clove-oil cannot be used with celloidin sections.

MOUNTING MEDIA.

Glycerine jelly and Canada balsam are commonly used for mounting purposes. For the laboratory balsam will be found a satisfactory medium. Should xylol be used for clearing, the balsam should be dissolved in xylol. Chloroform balsam may be used in sections cleared with chloroform.

For the formulæ of reagents and stains required for work in bacteriology and urinalysis, the reader is referred to the chapters in which is discussed the micro-technique of these subjects.

PART II.

NORMAL HISTOLOGY.

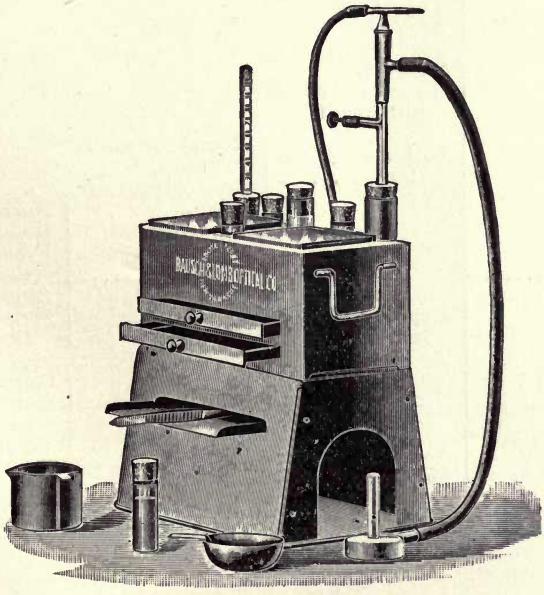
This brief discussion of the facts and principles of normal histology is applied chiefly to animal structures, with special reference to the human body. The animal body is composed of organs. Organs are constituted of tissues, and tissues consist of cells and intercellular substances. The histologist has to do chiefly with cells. Therefore, a thorough knowledge of the nature, structure, and functions of the cell is necessary to an adequate comprehension of this subject.

CHAPTER IV.

THE CELL.

The cell is a mass of protoplasm containing a nucleus and generally enclosed in a thin membrane called the cell-wall. The nucleus is believed always to be present, though there are some instances in which no nucleus has yet been discovered. The cell-wall is not an essential part, and is often absent; example, leucocytes and amœbæ.

Miller's Paraffin Bath.



Bausch & Lomb Optical Co., Rochester, N. Y.

OUTLINE OF THE CELL.

- **Structure**
  - Cell wall.
  - Protoplasm
    - Cytoplasm
      - Ectosarc.
      - Endosarc.
    - Nucleoplasm.
  - Centrospheres.
  - Nucleus
    - Nucleolus.
    - Chromatin.
  - Vacuoles
    - Water vacuoles.
    - Food vacuoles.
- **Cell contents**
  - Chromatophores
    - Chloroplasts.
    - Leucoplasts.
    - Chromoplasts.
  - Starch.
  - Mineral salts.
  - Proteids
    - Crystalloids.
    - Aleurone grains.
  - Fat.
  - Volatile oils.
  - Alkaloids.
  - Ferments.
- **Kinds of cells**
  - Vegetable.
  - Animal.
- **Functions**
  - Support.
  - Reservoirs of nutriment.
  - Cell multiplication
    - Normal fission.
    - Budding.
    - Free cell formation.
    - Karyokinesis.
  - Reproduction of individuals
    - Asexual
      - Normal fission.
      - Budding.
      - Free cell formation.
      - Rejuvenescence
      - Spore formation.
    - Sexual
      - Conjugation.
      - Parthenogenesis.
      - Fertilization.
  - Metabolism
    - Anabolism.
    - Katabolism.

STRUCTURE OF THE CELL.

The parts of a cell are the cell-wall, protoplasm, centrospheres, nucleus, and vacuoles.

(1) **The cell-wall.**—This is the thin membrane inclosing the protoplasm. With animals, the cell-wall consists of protein; with plants, it is composed of cellulose. Protein is a compound possessing the same elements as starch, but with nitrogen added. Cellulose, $C_6H_{10}O_5$, is an isomeric form of starch, having the same composition, but differing from it in being homogeneous in structure, not granular, and in being less easily dissolved and less readily converted into dextrin.

(2) **Protoplasm.**—This is the living substance of the cell, the organic basis of life. It contains carbon, hydrogen, oxygen, nitrogen, sulphur, and sometimes phosphorus, iron, and other elements. No chemical formula can be given for its composition, but it consists of unstable, constantly changing molecules. When dead, its chemical nature is changed, and it consists of protein, carbo-hydrates, water, and mineral salts. Of these, protein alone possesses nitrogen. Living protoplasm is irritable, unstable, deoxidizing; has the power to eliminate carbon di-oxide, and can reproduce itself, and, by assimilation, manufacture the innumerable products characteristic of plants and animals. The protoplasm of adjacent cells is sometimes connected by delicate threads, which pass through their walls. Protoplasm is a viscid, transparent substance resembling egg-albumen. It is never completely fluid. It is not homogeneous, but somewhat complicated in structure. It consists of two parts, the *cytoplasm* and the *nucleoplasm*. The cytoplasm constitutes the bulk of the protoplasm in the cell. It consists of an outer, dense film, the *ectoplasm,* and an inner, semi-liquid portion, the *entoplasm,* containing a fibrous sponge-work holding in its threads the *microsomes, centrospheres,* and *nucleus*. Microsomes are minute spherical masses supposed to contain nutrition for the growing cell, but their real functions are not well understood. The neucleoplasm enters into the structure of the nucleus and *nucleolus*.

(3) **The centrospheres.**—These are minute bodies associated with the nucleus and scarcely larger than the microsomes. Each centrosphere consists of an outer, hyaline film of *cytoplasm,* within

which is a fibrous sponge-work containing a dense body, the *centrosome.* The centrosphere multiplies by division, and thus initiates the complicated processes by which one cell develops into two.

(4) **The nucleus.**—This is the larger, rounded, dense portion of protoplasm. Its protoplasm is styled nucleoplasm. Its structure is similar to that of the cytoplasm, consisting of an outer, dense ectosarc and an inner sponge-work containing one or more nucleoli and the *chromatin,* a substance very susceptible to stains. The nucleus and centrospheres constitute the centers of vitality, the sources of growth and vital phenomena.

(5) **The valcuole.**—This is the cell cavity and contains a watery fluid or food masses for the nourishment of the cell. There may be several vacuoles in a cell. In very young cells there is no vacuole, the protoplasm filling the entire space. Old cells lose their protoplasm and may be empty or filled with the products of assimilation.

With plant cells the cytoplasm often forms a layer within the cell-wall, called the primordial-utricle.

CELL CONTENTS.

Besides the structures above named, the cell contains the chromatophores, starch, mineral salts, proteids, fat, volatile oils, alkaloids, ferments, and pigments.

(1) **The chromatophores** are the color bearers. Among plants there are three kinds—the *chloroplasts, leucoplasts,* and *chromoplasts.* The chloroplasts contain the green coloring matter, called *chlorophyl.* Some animals contain chlorophyl, as, for example, the Green Euglæna. Chlorophyl has the power to decompose carbon-di-oxide. Its chemical composition is unknown. It is soluble in alcohol. The chromoplasts are the proteid products which contain the coloring matter that gives the color to flowers and fruits. The leucoplasts, or amyloplasts, are corpuscles which engage in the manufacture of starch granules. They are found in portions of a plant removed from light.

(2) **Starch,** $C_6H_{10}O_5$, is found in the cell and is produced within the chloroplasts by the union of carbon (obtained from CO_2) and water. Reaction:

$$CO_2 = C + 2O$$
$$6C + 5H_2O = C_6H_{10}O_5$$

This process is carried forward under the influence of sunlight. Thus the plant stores up the energy of the sunbeam in complex molecules to be utilized by man and other animals.

Other carbo-hydrates often occurring in a cell are dextrin, glucose, cane sugar, etc.

(3) **Mineral salts.**—These often occur in a cell in crystalline form. Crystals in plant cells are called raphides.

(4) **Proteids.**—These occur in plant cells as crystalloids and aleurone grains. Crystalloids are protoplasmic bodies, crystalline in form, but not in character. Aleurone grains are proteid granules generally associated with crystalloids.

(5) **Fat** occurs in the cell as globules. It contains the same elements as starch, but the hydrogen and oxygen are not in the proportion of water. It serves as a reserve food supply.

(6) **The volatile oils,** such as turpentine, bergamot, and asafetida, occur especially in plant cells, and give to plants their perfumes.

(7) **The alkaloids** are organic bases bearing nitrogen. The solid alkaloids contain oxygen, whereas those that are liquid and volatile do not. When reacting with acids they form soluble salts. They furnish many powerful poisons and useful medicines, and are characteristic of plants.

(8) **The ferments** are nitrogenous compounds which have the power to bring about important chemical changes in organic substances.

KINDS OF CELLS.

The important kinds of plant cells are *parenchymatous* and *prosenchymatous* cells, *tracheids,* and *vessels.* Some of the varieties of animal cells are *leucocytes, epithelial cells, cartilage cells, bone corpuscles, marrow cells, lymphoid cells,* etc.

FUNCTIONS OF CELLS.

The cell is the laboratory of the body in which are manufactured all those complex products which enter into its structure. The processes by which these products are elaborated are *anabolism* (building up) and *katabolism* (breaking down). The two proc-

esses together constitute *metabolism,* the former being constructive metabolism and the latter destructive metabolism.

The cell, by the rigidity of its walls or its contents, gives support to the structures of the body.

The cell often serves as a reservoir for the products of assimilation.

The cell is the agent by which new cells are formed and by which the plant or animal is reproduced.

The method by which new cells are formed is a process of division. There are four forms of cell division—viz., *normal fission, budding, free cell formation,* and *karyokinesis.*

Normal fission is the simple division of a cell in which the protoplasm divides and a partition is formed between the two halves. Sometimes it takes place by a constriction of the cell-wall.

Budding.—This consists in the formation of a rounded projection, or bud, on the wall of the parent cell. This bud develops to normal size, becomes cut off by a partition, and generally separates from the original cells; example, yeast.

Free cell formation takes place when the protoplasm of the cell separates into one or more distinct masses, each mass forming for itself a cell-wall. The new cells are finally set free by the bursting of the wall of the parent cell.

Karyokinesis.—This is a form of fission in which the cell undergoes a cycle of changes, eventually producing two cells from one. It is more common among animals than plants.

For the study of karyokinesis the growing tips of onions and the larvæ of salamanders may be used. The cells may be fixed with Flemming's solution or chromic acid. Stain by the usual methods.

The different stages through which the cell passes in karyokinesis are: Resting nucleus, the skein, the rosette, the aster, the diaster, daughter rosettes, daughter skeins, and daughter nuclei.

REPRODUCTION.

Plants and animals reproduce *asexually* and *sexually.*

(1) **Asexual reproduction.**—This method is usually accomplished by the individual cell, and there are, therefore, no distinctions of sex, the new cells formed being exactly like the parent cell.

Often spores are formed which differ from the parent cell, but the spores are evidently asexual, neither being produced by sexual organs nor presenting sexual characteristics.

The varieties of asexual reproduction are the following:

(*a*) *Normal fission,* in which a unicellular animal simply divides into two; example, bacteria and amœbæ.

(*b*) *Budding.*—In this the unicellular organism produces a bud which eventually becomes cut off, forming a new individual; example, yeast.

(*c*) *By endospores.*—In this method new cells are formed within a large cell; example, lichens.

(*d*) *Rejuvenescence.*—By this process the protoplasm assumes a rounded mass, escapes from the cell-wall, and forms for itself a new cell-wall; examples, spirogyras and diatoms.

(*e*) *Spore-reproduction.*—The spore is a modified cell, whose function is to perpetuate and reproduce the species. Spores are generally formed in a spore case, or sporangium. The structural elements of the spore are the *exosporium,* or outer coat; *endosporium,* or inner coat; and the protoplasm.

(**2**) **Sexual reproduction.**—This occurs when one or two *sexual* cells engage to reproduce a plant or animal. Among plants the sexual elements are the spermatazoids and the oöspheres. The sexual elements of animals are the spermatazoa and the ova. The varieties of sexual reproduction are conjugation, parthenogenesis, and fertilization.

(*a*) *Conjugation.*—This consists in the union of two like cells. In some cases the protoplasm of one cell is discharged into that of another, the resulting cell being called a zygospore, or auxospore; example, Spirogyra. It may occur, also, by the union of the protoplasm of two distinct cells which have previously discarded their cell-walls. In this method the cells are structurally the same, but as the process is analagous to that of the sexual method—that is, cytoplasm fusing with cytoplasm, and nucleus with nucleus, it is considered by the best authorities as sexual in character; examples, diatoms and animalcules.

(*b*) *Parthenogenesis.* — This occurs when one sex alone produces a new individual. A single sexual cell may be concerned or

two cells of the same sex. It is illustrated among aphides and certain of the humbler lepidoptera.

(*c*) *Fertilization.*—This occurs when two sexual cells unite to produce a new individual. It is a process common to higher plants and animals.

It is evident, therefore, that each individual plant or animal has its origin in a single cell, and that its organism is developed by a process of cell-multiplication. For example, by the fusion of the sexual elements the primordial cell of the *animal* body is formed. This cell divides by a process of karyokinesis and forms two cells. Each of these divides, similarly, forming four cells; the four likewise produce eight; and the eight produce sixteen. This gives what is called the morula, or mulberry stage. By rearrangement of these cells in the form of a pouch there is formed the gastrula, the cells disposing themselves in two layers. From these layers is produced a middle layer, thus forming the blastoderm, consisting of three distinct layers,—epiblast mesoblast, and hypoblast. From these layers, by cell-multiplication, growth and differentiation, all the structures of the body are produced.

Laboratory exercise No. 4.—*The structure of a cell.* Peel from the outer surface of a scale of an onion bulb a piece of the epidermis. Apply to a slide and stain for a few moments with rosanilin violet. Wash with water, apply cover-glass, and examine. Observe first the form of the cells and their relative positions, then the structure of each cell. Make out the cell-wall, the nucleus with its nucleolus, and the granular protoplasm surrounding it. Do you observe any vacuoles? Make a drawing of several cells, exhibiting the structures above named.

Laboratory exercise No. 5.—*To demonstrate protoplasm and cellulose.* Make a preparation similar to the above and apply a drop of iodine solution. Examine. The protoplasm will be stained brown, but the cell-wall is not stained. Now remove cover-glass and apply a small drop of sulphuric acid. This changes the cellulose into soluble dextrin, which is attacked by the iodine and turned blue or black. This is the iodine test for starch. Look for crystals of iodine and for the Brownian movement among the molecular particles.

THE BROWNIAN MOVEMENT.

This is a molecular movement purely physical in character. It occurs among bacteria and may be mistaken for independent motion due to vitality.

A STUDY OF CELLS.

In the following studies it is designed to illustrate the different forms of cells and the methods of cell-multiplication. Types are presented which are believed to have a special bearing on the work of the histologist. Yeast, Protococcus, and Spirogyra have been selected to illustrate plants, and the Amœba, Green Euglæna, and Slipper Animalcule, to illustrate animals.

STUDY OF THE YEAST PLANT.

The Yeast Plant is a unicellular, chlorophylless saprophyte, which reproduces by budding and ascospores.

Classification:

Kingdom—Vegetable.
Series—Cryptogamia.
Sub-kingdom—Thallophyta.
Class—Fungi.
Sub-class—Ascomycetes.

Life History and Morphology.—The Yeast Plant, *Saccharomyces cervisiæ*, is the common species used by brewers and bakers. It consists of cells, round or oval in outline. Each plant, or cell, is called a *torula;* when the cell produces spores the term *gonidia,* or *ascospores,* is applied to them, while the term *ascus* is applied to the cell. The cell-wall is transparent and composed of cellulose. The protoplasm contains one or more clear spots (vacuoles), and is believed to contain a nucleus. Multiplication occurs by budding. A spherical projection, or papilla, is produced on the wall of the parent cell, which forms for itself a cell-wall, and, eventually, by a partition, becomes separated and assumes an independent existence. Before separation occurs, however, owing to rapid growth, the daughter cells often throw out buds, thus forming a colony or chain. Reproduction also occurs by the formation of endospores. The protoplasm of the parent cell divides into four masses, each of which forms a new cell-wall. These are called ascospores and have the power to perpetuate the plant under unfavorable conditions. By the dissolution of the wall of the parent cell the ascospores are set free and, under favorable conditions, reproduce the plant.

In size, the yeast plant ranges from $\frac{1}{4000}$ to $\frac{1}{2500}$ inch in diame-

ter. In form, the torula is spherical or ovoidal. It has the power to produce fermentation, thus changing sugar into alcohol and CO_2.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

Some of the sugar breaks up into glycerine and succinic acid Yeast contains no chlorophyl, and, therefore, has not the power to decompose CO_2. The elements necessary to form its protoplasm, cellulose, fat, etc., are carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorus, potassium, magnesium, and calcium. These are obtained chiefly from complex substances, which are broken up by its action, and their elements appropriated to form the new compounds required for its growth and reproduction.

Saccharomyces cervisiæ is used in making bread, the CO_2 formed permeating the dough and making it spongy. Several species are used in making beer and other fermented liquors, different flavors being produced by different species.

Laboratory exercise No. 6.—*Yeast.* Place in a test tube, half filled with water, a small portion of a cake of Fleischman's yeast, and let stand for a few hours in a warm place. Place a drop of the liquid upon the slide; cover and examine with H. P. Look for single cells; then for a large cell with a small one attached; the small cell is a bud. Find a group of cells; this is a colony. Look also for a chain. Irrigate with magenta, and search for protoplasm and a nucleus.

Upon the freshly cut surface of a potato sow some yeast cells. In a day or so examine some of the growth and search for ascospores. They will appear as rounded masses within the larger cells. Make drawings to illustrate a single cell, a colony, a chain, buds, and ascospores.

PROTOCOCCUS.

Protococcus is a unicellular, chlorophyl-bearing plant which reproduces by normal fission and by endospores.

Classification:

Kingdom—Vegetable.
Series—Cryptogamia.
Sub-kingdom—Thallophyta.
Class—Algæ.
Sub-class.—Chlorophyceæ.
Order—Protocaccales.
Family—Protococcaceæ.
Genus—Protococcus.
Species—*Protococcus vulgaris.*
Protococcus pluvialis.
Protococcus nivalis.

Life History and Morphology.—Protococcus vulgaris, Green Protococcus, is a spherical organism ranging in size from 1-10,000 to 1-350 of an inch in diameter. It consists of a cell-wall (cellulose), protoplasm, nucleus, and nucleolus. Within the cell the protoplasm is in green-colored masses containing chlorophyl. These are the *chromatophores,* or chloroplasts. Multiplication takes place by fission. The protoplasm divides first; then a partition is formed between the two portions, and the cells thus formed separate. Often, however, before the new cells separate, one or both may again divide, thus forming groups of three or four cells; these also may be subject to fission, so that groups of six, eight, etc., may occur, the number generally being some multiple of two. There are two states of protococcus: The quiescent, just described; and the motile form, which is ovoidal in shape and is provided with two *flagella* which, by their contraction, give to the cell a whirling motion. This state of protococcus is called a *zoöspore.* In course of time the zoöspore loses its flagella and becomes a quiescent cell.

Protococcus pluvialis is found in the gutters of houses, and differs from the foregoing in possessing a small amount of red pigment. It, also, reproduces by endospores. The protoplasm of the resting cell divides into four portions or into many portions, in the former case producing *megazoöspores,* and in the latter, *microzoöspores.* These are set free by the bursting of the wall of the parent cell.

Protococcus nivalis is the so-called red snow of Arctic regions.

Habitat.—Protococcus vulgaris is found on brick, rocks, fences, houses, and the bark of trees.

Laboratory exercise No. 7.—*Protococcus.* From the north side of a tree or fence obtain some bark or wood containing a coating of green Protococcus. Moisten and place under a bell jar or in a Petri dish for twenty-four hours in a warm place. When the cells have begun to vegetate, apply the surface of a cover-glass. Some of the cells will adhere. Add a small drop of water if necessary, and examine with H. P. Observe the shape, color, and size of the cells. Find a single cell; then one with a partition showing normal fission. Find also a triplet and groups of four, eight, etc. Search now for motile forms. These are the zoöspores. Irrigate your preparation with acetic acid, and discover, if possible, a nucleus. Make drawings illustrating a single cell, doublets, triplets, and groups of four or more; also zoöspores.

SPIROGYRA.

Spirogyra is a unicellular, chlorophyl-bearing plant which reproduces by normal fission and by conjugation.

Classification:

Kingdom—Vegetable.
Series—Cryptogamia.
Sub-kingdom—Thallophyta.
Class—Algæ.
Sub-class—Chlorophyceæ.
Order—Conjugatales.
Family—Zygnemaceæ.
Genus—Spirogyra.
Species—*Spirogyra nitida.*
Spirogyra maxima.

Life History and Morphology.—Spirogyra maxima, commonly called Brook Silk, consists of a cylindrical cell, longer than broad. The cells, placed end to end, are united into long filaments by a gelatinous secretion. They contain chlorophyl bodies arranged in spiral form, hence the generic name. These are the *chromatophores,* or *chloroplasts.* A nucleus is always present, but not easily seen. Fission occurs by the normal method and may take place in any cell of a filament. Reproduction also takes place by conjugation—i. e., two adjacent cells of filaments lying near, or in contact with each other, unite by the protoplasm of one being discharged into that of the other. This is accomplished by tubular projections being thrown out from each cell, which meet and form a passageway between the cells. In this case the cells are alike and distinction of sex has not yet been discovered. The new cell produced by the conjugation is called a *zygospore.* The encysted zygospore becomes embedded in the mud and preserves the life of the plant through the winter. It develops into a new plant by the protrusion of the inner coat through the broken outer coat, when fission takes place, and a filament is produced by the vegetative process. By some, spirogyra is supposed to illustrate sexual reproduction, the conjugating cells being the gametes and producing by their union the zygospores. The sexual character of the cells may yet be proven, for there may be

sexual differences (physiological at least) of which we are yet ignorant. The chromatophores often contain bright spots, called *pyrenoids,* and are instrumental in producing starch, which, as minute granules, occurs surrounding the pyrenoids. The cell exhibits cell-wall, primordial-utricle, protoplasm, nucleus, chromatophores, starch grains, pyrenoids, and chlorophyl.

Habitat.—Spirogyra may be found in ponds and slow-running creeks, and is widely distributed. It is somewhat smooth and slimy to the touch.

Laboratory exercise No. 8.—Obtain from some pond or brook a quantity of Spirogyra, placing it in a large jar with water. Examine with H. P., observing the filaments composed of cells attached end to end. Make a study of a single cell. Observe the spiral arrangement of the chloroplasts. Use the 1-12-inch objective and search for the pyrenoids and starch granules. Find cells in the process of fission. This is to be observed where the cells are much shorter than normal size. Irrigate with a drop of acetic acid, and search for a nucleus. Make drawings to illustrate filaments and a single cell containing nucleus, chloroplasts, and cell-wall.

A STUDY OF ANIMAL CELLS.

AMŒBA.

The Amœba is a unicellular animal devoid of a cell-wall, having the power to produce pseudopodia, and reproducing by normal fission.

Classification:

Kingdom—Animal.
Series—Protozoa.
Sub-kingdom—Protozoa.
Class—Monera.
Order—Amœbea.
Genus—Amœba.
Species—*Amœba proteus.*

Life History and Morphology.—The Amœba is an animal which appropriates food without a mouth, digests without a stomach, breathes without lungs, and has sensation without a nervous system. It is simply a mass of protoplasm consisting of cytoplasm and

nucleoplasm. The cytoplasm consists of two layers, the ectoplasm, or dense, outer hyaline layer, and the endoplasm, or inner layer, containing the microsomes. The nucleoplasm constitutes the nucleus and contains masses of chromatin, which is very susceptible to staining reagents.

The animal has the power to throw out protrusions, or *pseudopodia,* from its body. A bulb is formed on the surface of the cytoplasm, and then all the protoplasm flows in this direction until, often, the whole animal has passed into the pseudopodium. In the meantime, other pseudopodia are produced. The movement exhibited by the leucocytes of the blood is similar to this and is called the amœboid movement. Food is obtained by flowing around it. There are two kinds of vacuoles, *food vacuoles* and *water vacuoles.* The *contractile vesicle* serves the function of excretion. The Amœba reproduces by normal fission—that is, the nucleus and protoplasm divide into two masses, without undergoing any process of mitosis. There are two stages of this animal, the active stage, just described, and the encysted, or quiescent, state. When the animal is placed under unfavorable conditions it contracts into a sphere, forms an enclosing tough membrane, and in this condition may be wafted by the air from place to place. Under favorable conditions the protoplasm breaks through the enclosing wall, and the animal again enters the active stage.

Habitat.—The Amœba may be found in ponds or brooks upon submerged leaves and stems, or in the ooze which collects at the bottom. To obtain material for laboratory use, collect some grass and leaves from the side of a stream, also some submerged plants and ooze from the bottom of a pond or stream, and place in glass jars for five or six weeks. At the end of this period amœbæ should be obtained in numbers.

Laboratory exercise No. 9.—*Amœbæ.* From the infusion, prepared as directed, carefully pick out a submerged leaf, and apply some of the ooze upon its surface to a slide. Cover and examine with L. P. and H. P. Observe the ectoplasm, endoplasm, and nucleus. Make out the vacuoles and contractile vesicles. Observe the formation of pseudopodia. Make drawings to illustrate protoplasm, nucleus, pseudopodia, and encysted stage.

THE SLIPPER ANIMALCULE.

The Slipper Animalcule may be found in hay infusion. It is a unicellular animal, provided with a cell-wall, and reproduces by normal fission.

Classification:

Kingdom—Animal.
Series—Protozoa.
Sub-kingdom—Protozoa.
Class—Infusoria.
Order—Ciliata.
Genus—Paramœcium.
Species—*Paramœcium candatum.*

Life History and Morphology.—The Slipper Animalcule is a single cell provided with cell-wall, protoplasm, a double nucleus *(macro-nucleus* and *micro-nucleus),* two *contractile vesicles,* and food and water *vacuoles.* It receives its food through a mouth. Leading to the mouth is an *œsophagus* which terminates near the middle of the body surface in a depression called the *vestibule.* This depression, as well as the whole surface of the cell, is provided with cilia. The cilia are minute, hair-like, protoplasmic projections which by their rapid motion enable the animal to move from place to place. Excretion is accomplished by means of an *anal spot.* This is not a permanent opening, but a thin place in the cell-wall through which the excretions are forced by means of the contractile vesicles. Reproduction occurs by normal fission, and is accomplished by the division of the nucleus and protoplasm into two halves and the constriction of the cell-wall.

Laboratory exercise No. 10.—*Slipper Animalcule.* **Examine some of the scum which forms on the surface of hay infusion. Examine with the high power, and, having found a specimen of the animalcule in a quiet condition, observe the following structures: Cell-wall, cilia, vestibule, œsophagus, mouth, protoplasm, double nucleus, food masses, the water vacuoles, and the contractile vesicles. Make a drawing to illustrate the structures thus named.**

GREEN EUGLÆNA.

Green Euglæna is a unicellular animal which possesses chlorophyl, secures its motion by means of flagella, and reproduces by normal fission.

Classification:

Kingdom—Animal.
Series—Protozoa.
Sub-kingdom—Protozoa.
Class—Infusoria.
Order—Flagellata.
Genus—Euglæna.
Species—*Euglæna viridis.*

Life History and Morphology.—Euglæna viridis is an interesting animal because of the fact that its cell-wall is composed of cellulose and because it possesses chlorophyl. The cell is fusiform in shape, and is provided with *cell-wall, mouth, flagellum* (extending from an anterior depression), an *eye spot* of red pigment situated near the mouth, and *protoplasm, chloroplasts,* and *nucleus.* It has a rotary motion. Fission takes places in a longitudinal direction instead of transversely, as with the Slipper Animalcule.

Habitat.—The Green Euglæna may be found in damp soil, in sewerage, and upon the surface of stagnant water.

Laboratory exercise No. 11.—*Green Euglaena.* Obtain some Green Euglæna, preferably from the surface of water. Examine with the high power. Make out carefully the structures mentioned above. Do you find any evidences of cilia? In what two respects does Euglæna resemble a plant? What is your conclusion as to its nature? Drawings.

VEGETABLE CELLS.

A. Onion Cell. **B. Yeast.**

A. Onion cell: (a) Cell-wall: (b) Cytoplasm; (c) Primordial utricle; (d) Nucleus; (e) Nucleolus; (f) Vacuoles.

B. Yeast plant: (a) Single cell; (b) Cell wall; (c) Protoplasm; (d) Buds; (e) Colony; (f) Chain; (g) Ascospores.

A. Protococcus. **B. Spirogyra.**

A. Protococcus: (a) Single cell; (b) Cell wall; (c) Chloroplasts; (d) Cell division; (e) Groups of three, four, six, etc.; (f) Zoöspores; (g) Flagella.

B. Spirogyra: (a) Single cell; (b) Cell wall; (c) Spiral of chloroplasts; (d) Nucleus; (e) Pyrenoids; (f) Starch granules; (g) Cell division; (h) Conjugation.

ANIMAL CELLS.

Amœba.

Amœba: (a) Active stage; (b) Cytoplasm; (c) Nucleus; (d) Pseudopodia; (e) Food vacuoles; (f) Water vacuoles; (g) Contractile vesicle; (h) Microsomes; (i) Normal fission; (j) Encysted stage.

A. Slipper Animalcule. **B. Green Euglæna.**

A. Slipper Animalcule: (a) Cell-wall; (b) Cilia; (d) Vestibule; (e) Œsophagus; (f) Mouth; (g) Protoplasm; (h) Macronucleus; (i) Micronucleus; (j) Food masses; (g) Vacuoles; (h) Contractile vesicles; (i) Normal fission.

B. Green Euglæna: (a) Cell-wall; (b) Chloroplasts; (c) Mouth; (f) Eye-spot; (g) Flagellum.

CHAPTER V.

TISSUES AND ORGANS.

A tissue consists of intercellular substance and an aggregation of cells of common origin which usually exhibit a common form, structure, and function. The intercellular substance may be very slight in quantity (merely a delicate layer of cement between the cells, as in epithelial tissue), or it may make up the bulk of the tissue, as illustrated in the calcareous deposit of osseous tissue. It is deposited by the cells and is usually formed by their agency. In some tissues the cells vary in form; for example, in epithelial tissue, the newly-formed cells are almost spherical and are rich in protoplasm, while the old cells are merely flattened scales devoid of protoplasm. In osseous and nervous tissues the young cells may be spherical or oval, while the older cells are provided with protoplasmic processes.

An organ is a single tissue which exhibits a special function or a group of tissues so associated as to accomplish some definite purpose in the plant-or animal economy. It is often the case that an organ may serve several purposes; as, for example, the tongue, which aids in mastication, deglutition, and articulation, and is an organ of taste and secretion. There is usually, however, one function which is preëminent. In the structure of an organ it is not the rule that all its tissues are derived from the same source. The tissues of the stomach, for example, are derived from the epiderm, mesoderm, and hypoderm.

As has been suggested in the preceding chapter, all the organs and tissues of the animal body are derived from a single cell. This cell is produced by the fusion of a sperm nucleus with a germ nucleus. From this primordial cell, by a process of segmentation, there is produced, first, the morula; then the gastrula, with its two layers—epiblast and hypoblast. From these layers is derived a third layer, the mesoblast. We now have the blastoderm, consisting of three distinct layers of cells—epiblast, mesoblast, and hypoblast. From these layers are produced the primitive layers of the embryo—epiderm from the epiblast, mesoderm from the meso-

blast, and hypoderm from the hypoblast. From these primitive tissues are derived all the structures of the body.

From the epiderm are developed the nervous system, the epithelium, covering the surface of the body, the enamel, the nails, the hair, the organs of special sense, and all glands except those which open into the alimentary tract from the œsophagus downward.

From the mesoderm are derived the blood, blood-vessels, all the connective tissues (cartilage, bones, tendons, etc.), the muscles, the dentine, and cementum.

From the hypoderm are developed the epithelial lining of the alimentary canal and the glands opening into it.

Tissues may be classified into four groups: (1) Epithelial tissues, (2) Connective tissues, (3) Muscular tissues, (4) Nervous tissues. The organs of the body are constituted of these tissues. For example, the tongue consists of epithelial, muscular, connective, and nervous tissues. In the following studies tissues and organs are treated in the order considered most convenient for practical work. The first structure to be considered is the blood.

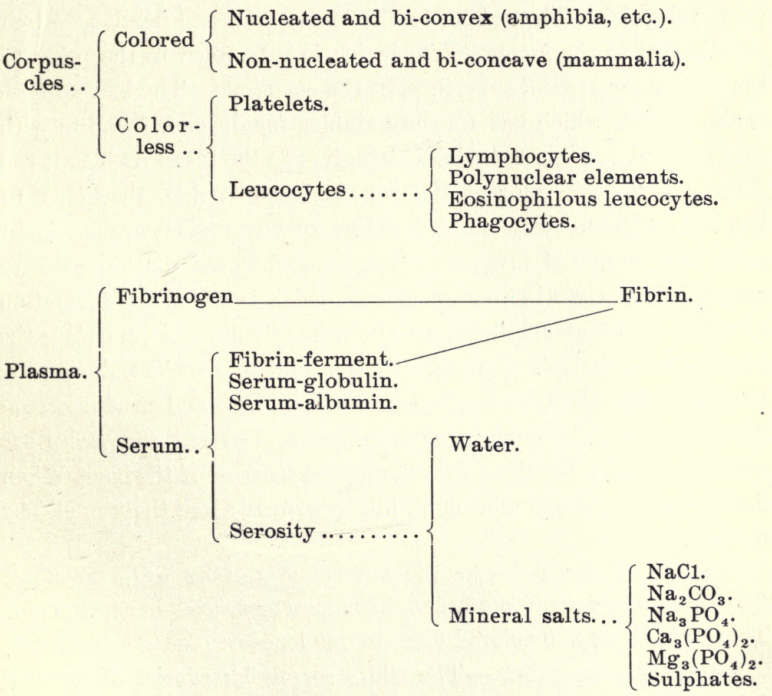
MEMORANDA.

CHAPTER VI.

THE BLOOD.

The blood is a tissue of mesodermic origin. It consists of cells, called *corpuscles,* and an intercellular substance—the *plasma,* or *liquor sanguinis.* The following outline will exhibit its composition as well as its structural elements:

OUTLINE OF THE BLOOD.



The corpuscles are the cellular elements of the blood. In man the red corpuscles, being devoid of protoplasm, are not alive, but the leucocytes exist in the blood as so many distinct amœboid animals. They can produce pseudopodia, and have the power of reproducing themselves by normal fission and karyokinesis. The red

corpuscles occupy about fifty per cent of the volume of the blood in man, thirty-five per cent in woman. The volume of the white corpuscles is about two per cent.

Colored corpuscles. —In the blood of man these are usually styled red discs; as seen on edge, they appear to bulge at the extremities and have concave centers. They are devoid of cell-wall, and on account of their flexibility are capable of crowding through very narrow spaces.

In the fish, salamander, reptile, and bird the red corpuscle is nucleated and bi-convex. Among mammals, including man, it is bi-concave and non-nucleated. Under the microscope the color appears yellowish. The red corpuscles are smooth and flexible. Their color is due to the *hæmaglobin,* which is suspended in the pores of the *stroma,* or ground substance, of the corpuscle. The hæmaglobin contains iron, which has a strong affinity for oxygen, forming with that element *oxyhæmaglobin,* which gives to the blood its bright red tinge. The dark color of the venous blood is due, therefore, to the hæmaglobin. The chief function of the red corpuscles is to serve as a carrier of oxygen. When exposed to the air the red discs arrange themselves in rows or stacks, which is called the formation of rouleaux. Water will remove the hæmaglobin. Sirup causes the corpuscles to shrivel. Normal saline produces *crenation,* due to the fact that the salt has an affinity for the stroma and produces contractions upon the surface by exosmosis. The red corpuscles are manufactured in the liver, spleen, and red marrow of the bone. When first formed they are nucleated, but afterwards lose their nuclei by mitosis.

The size of the red disc in man is $\frac{1}{3200}$ of an inch, or about 7.5 μ. It would require 40,000 of them to cover the head of a pin. There are about 350 colored discs to one leucocyte.

Colorless corpuscles. —The white corpuscles consist of the *platelets* and *leucocytes.* The platelets are colorless elements, about $\frac{1}{4500}$ of an inch in diameter. They are sometimes very abundant. In man the leucocytes are spherical in shape, nucleated, and larger than the platelets and red disks. They are alive and exhibit the amœboid movement, throwing out pseudopodia, by means of which they move from place to place. There are four impor-

tant varieties. The *lymphocytes* are the mono-nucleur elements, and may be large or small, according as the nucleus more or less than half fills up the space of the cell. The *polynucleur leucocytes* possess more than one nucleus, sometimes four or five nuclei. The *eosinophilous leucocytes* are those which take the eosin stain. They do not act as scavengers in the system. The *phagocytes* comprise all leucocytes which are not stained by eosin, or about seventy-five per cent. They serve as scavengers in the body, removing fat and foreign substances from the blood and attacking and destroying obnoxious microbes. The leucocytes also assist in the process of anabolism, migrating through the stigmata of the capillaries to build up worn-out tissues and going to the relief of diseased structures. Leucocytes have no cell-wall.

The *plasma* is the liquid part of the blood and consists of the *fibrinogen* and the *serum*. The fibrinogen is a proteid compound which under the stimulus of the *fibrin-ferment* forms the *fibrin*. The fibrin is formed when the blood is exposed to air, heat, etc., solidifying in slender fibres which collect in their meshes the corpuscles, forming a clot. This process is known as coagulation. The liquid which oozes from the clot is the *serum*. The serum contains the *fibrin-ferment*, the *serum-globulin*, the *serum-albumen*, and the *serosity*. The first three of these are proteids and contain nutritive material for the growing cells. The serosity consists of the water and the mineral salts. The mineral salts commonly found in the blood are sodium carbonate, sodium chlorid, sodium phosphate, magnesium phosphate, calcium phosphate, and a small amount of sulphates.

The microscopic study of the blood is an important aid in determining the condition and relative number of its structural elements and the presence of invading parasites. The serum of the blood is believed to be germicidal, but under abnormal conditions it becomes infested with certain species of bacteria. A species of Vermes, *Distoma Hæmatobium*, and a protozoan, *Plasmodium malariæ*, are two important animal parasites which infest the blood.

Plasmodium malariæ.—This parasite is considered by some authorities to be a plant; by others it is placed (with other organisms) in a separate kingdom, called Protista. The view here adopted (that

it is an animal) is the one generally accepted. This organism attacks the red corpuscles, destroying the hæmaglobin. It may be stained with methylene-blue, aqueous or alcoholic solution, and can be detected as a minute body, irregular in shape, filling about one-fourth of the corpuscle. Its life history includes five distinct stages: (1) The spore; (2) the protoplasmic, or fission, stage; (3) the amœboid form; (4) the plasmodium, consisting of several amœboid forms united; (5) the encysted stage, containing a cell-wall within which the cell contents break up into spores. This cycle of changes through which the organism passes accounts for the fact that persons afflicted with malaria experience a recurrence of the symptoms at stated intervals.

Laboratory exercise No. 12.—*Corpuscles, rouleaux, and fibrils of fibrin.* Clean a slide and cover-glass. This step should precede nearly all the exercises that follow. Wrap the ring finger with a kerchief from the base to the first joint. Apply a few drops of alcohol to cleanse the exposed surface, and then, with a lance or sterilized needle, with a quick motion puncture the skin just above the root of the nail. Wipe off the first drop of blood, and to the next apply the surface of a cover-glass. Place this upon a slide, blood down. Now examine with H. P., observing first the red corpuscles, which will be found to be collecting by their flat surfaces into rows, called rouleaux. Examine one of the discs on edge, then in profile. With fine adjustment focus up and down. Why does the center of the disc appear alternately dark and light? Examine now the platelets and leucocytes. They may be found in the clear spaces between the red discs, and, as they adhere to the glass, do not float about in the serum. Lay aside this preparation until the next period and examine for the fibrils of fibrin, which will appear as delicate threads beneath the cover-glass.

Crenation and Amœboid Movement. Make a second preparation by the method above described. Add to the blood a small drop of normal saline. Examine and observe the crenated appearance of the colored discs. This is due to exosmosis. Gently warm the slide and look for the amœboid movement of the leucocytes. To observe this may require that the slide be kept warm for a considerable period.

Acetic acid brings into view the nuclei of leucocytes. Water removes the hæmaglobin from red corpuscles, while sirup causes the disc to shrivel.

Laboratory exercise No. 13.—*Preparation of a blood slide.* Secure a drop of blood by the method described, and apply a clean cover-glass. To this apply a second cover-glass, and with gentle pressure spread out the blood. Then, with a quick motion, keeping the cover-glasses parallel, draw them apart so as to leave a thin film of blood on each. Select the best preparation, lay it upon a piece of writing paper, blood up, and

hold it over a flame, being careful not to ignite the paper, until the film turns brown. The blood is now properly affixed, and may be stained by the following method:

No. XI. Scheme for Staining Blood Preparations.

(1) Make a cover-glass preparation and affix by the method given above.

(2) Using Cornet forceps, stain with alcoholic or glycerine eosin, thirty minutes to one hour.

(3) Wash off eosin by dipping cover-glass vertically into distilled water one or two times.

(4) Apply Delafield's hæmatoxylin fifteen minutes.

(5) Wash in water, dry with gentle heat, and mount in balsam.

(6) Label and study.

Observe that the colored discs are stained red by the eosin, while the nuclei of the leucocytes are stained blue by the hæmatoxylin. Find several lymphocytes and compare the sizes of their nuclei. Study the polynuclear elements. How many nuclei do you find? Make drawings to represent the red corpuscles on edge and in profile, rouleaux, crenation, fibrils of fibrin, lymphocytes, and polynuclear elements.

Laboratory exercise No. 14.—*Staining for Plasmodium malariae.*

(1) Make a cover glass preparation from a malarial patient by the usual method.

(2) Stain with alcoholic methylene blue, fifteen minutes.

(3) Wash in water and examine.

(4) Dry, mount and label, if a good specimen.

Search for the organism in the red corpuscles, also in the plasma. It has no definite form, but may be semi-lunar, spindle-shaped, spherical, etc. It can be recognized by its color, the discs not taking the blue stain.

Laboratory exercise No. 15.—*Counting the blood corpuscles.* Probably the most satisfactory device for counting the corpuscles is by means of the centrifuge. Pursue the following method: Attach to the graduated blood tube a piece of rubber tubing. Having secured a large drop of blood, fill the graduated tube by gentle suction, and then place it in the hæmatokrit, making the bearings secure. Revolve the handle of the centrifuge seventy-seven times in one minute. This will give 5,000 rotations of the hæmatokrit, resulting in the precipitation of the red corpuscles to the outer end of the tube, the leucocytes being arranged next, and the plasma filling the other end. If the red corpuscles fill half the tube, standing at the graduation marked "fifty," it indicates that there are 5,000,000 corpuscles in a cubic millimeter of the blood; if it stands at the mark "thirty-five," there are 3,500,000 in a cubic millimeter. The numbers given above indicate the normal amount of corpuscles for man and woman respectively. Should there be less than the normal number, it indicates anæmia. Should there be the required number of red disks, but too many leucocytes, there is an indication of leukæmia. What other method may be employed for counting corpuscles?

BLOOD.

Structural Elements.

Blood: (a) Red discs in profile showing dark and light centers; (b) Disc on edge; (c) Rouleaux; (d) Crenation; (e) Platelets; (f) Leucocytes; (g) Nucleus; (h) Amœboid movement; (i) Fission; (j) Fibrils of Fibrin; (k) Crystals of hæmatin.

A. Leucocytes. **B. Plasmodium malariæ.**

A. Leucocytes: (a) Large lymphocyte; (b) Small lymphocyte; (c) Polynuclear elements.

B. Plasmodium malariæ: (a) Corpuscle; (b) Plasmodium; (c) Spores.

CHAPTER VII.

ENDOTHELIUM AND EPITHELIAL TISSUES

Endothelium. —This structure is of mesodermic origin and consists of a single layer of very thin polyhedral cells united edge to edge by a cement substance. It lines surfaces not directly exposed to the external atmosphere, such as the surfaces of serous and synovial membranes, forming the linings of the heart, blood tubes, and other organs.

EPITHELIUM.

Epithelium is derived from the epiderm and hypoderm of the embryo. It consists of cells of various shapes, which are united by cement and are devoid of blood-vessels. The important functions of epithelium are protection, secretion, and elimination. The shape of the cells depends upon the amount and kind of pressure exerted upon them. As a rule, when first formed, they are spherical. New cells are produced by karyokinesis. Blood vessels being absent, nourishment takes place by absorption.

The following are the important varieties:

Squamous.—*Simple and Stratified.*

Columnar.—*Simple and Stratified.*

Ciliated.—*Simple and Stratified.*

Modified.—*Goblet, Pigmented, and Transitional.*

Specialized.—*Glandular and Neuro-epithelium.*

Squamous epithelium.—This structure consists of irregular, polyhedral, flattened cells, united edge to edge in the form of a pavement. The cells, when seen on edge, are found to be somewhat biconvex. The nucleus is somewhat eccentric. Squamous epithelium is found wherever surfaces are subjected to considerable friction. There are two varieties—viz., simple and stratified. Simple squamous epithelium consists of a single layer of cells and is found lining the air cells of the lungs, the capsule of the Malpighian body, the descending limb of Henle's arch, parts of the brain-ventricles, and a few other places.

The stratified variety consists of several layers and is found cov-

ering the skin, mouth, tongue, lower half of pharynx, œsophagus, epiglottis, upper part of larynx, pelvis of kidney, ureter, bladder, beginning and end of male urethra, and the whole female urethra.

The deeper layers of this tissue have cells more nearly spherical, which are often connected with each other by slender processes. These processes give rise to the so-called prickle cells of the deeper layers of the epidermis. The cells of the outer layer become much flattened, lose their protoplasm, and by constant friction are worn away and cast off. This process is called desquamation.

Columnar epithelium.—This form of epithelial tissue is constituted of cells which are columnar in shape as seen from the side, but from above they appear hexagonal. The first layer, resting upon the *membrana propria,* consists of spherical cells, but those of the next layer are oval. Simple columnar epithelium is found lining the mucous membrane of the alimentary tract from the cardiac orifice downward. The stratified variety is found in the excretory ducts of glands leading into the alimentary tract and in a portion of the male urethra. This tissue is of hypodermic origin.

Ciliated epithelium.—This resembles columnar epithelium, but the outermost layer of cells is provided with cilia. Cilia are delicate protoplasmic projections which by their motion produce outward currents of mucus and other products. The cells are nucleated and rest upon a basement membrane. Ciliated epithelium occurs in the nasal cavities, the Eustachian tubes, larynx, trachea, bronchi, a portion of the uterus, Fallopian tubes, vasa efferentia (partly), the ventricles of the brain, and the central canal of the spinal cord.

Modified epithelium.—This is represented by modifications of the types given above. The important varieties are goblet cells, pigmented epithelium, and transitional epithelium.

Goblet cells are modifications of columnar or ciliated cells. Each cell is generally isolated from others of like character and is formed by the elaboration of mucin from the protoplasm, which so fills up the cell as to cause it to become swollen and elliptical in shape. Eventually the cell bursts, discharging its contents upon the surface of the membrane. This is one source of mucus, and hence the term *mucous* membrane.

Pigmented epithelium.—This is represented by cells of the squa-

mous type which have become impregnated with melanin, a dark pigment that gives coloration to the structure. The pigmented epithelium of the retina is the best illustration.

Transitional epithelium.—This occurs in the urinary tract and is illustrated by modifications of squamous and columnar cells, where the one kind merges into the other. It occurs in the pelvis of the kidney, ureter, bladder, and urethra. The cells are round, spindle-shaped, cuboidal, or pear-shaped, and often exhibit one or more slender processes.

Specialized epithelium.—This form of epithelial tissue consists of cells so specialized as to engage in the elaboration of secretions; or to perform some special function. There are two varieties—glandular epithelium and neuro-epithelium.

Glandular epithelium.—The terms *cuboidal* and *secretory* also apply to this tissue, the former arising from the general shape of the cells, the latter from their functional character. It occurs in the intestinal, gastric, and salivary glands, the pancreas, and liver.

Neuro-epithelium.—This comprises highly specialized cells which aid in nerve sensation. They are found at the terminations of the nerves of special sense. The cells are generally elongated and contain an inner nuclear part and an outer part directed toward the periphery, which is often provided with hair-like processes. The rods and cones of the retina and the olfactory and taste cells are illustrations.

The following ten characteristics of epithelial cells should be carefully considered: (1) The cells are superficially disposed; (2) they are united by cement; (3) they contain no blood-vessels; (4) they vary greatly in shape; (5) they perform various functions, those of protection and secretion being the more common; (6) the cells multiply by karyokinesis; (7) they are nourished by absorption; (8) they have eccentric nuclei; (9) they rest upon a basement membrane, or membrana propria; (10) the cells contain mucin, melanin, etc. It should be borne in mind that all these characteristics are not universally present.

Laboratory exercise No. 16.—*Study of epithelial cells.* Collect upon the end of the tongue a quantity of saliva and apply the same to the center of a slide. Cover and examine with high power. Observe the

cells, scarcely visible, and note the protoplasm, nuclei, and nucleoli, also the cell-wall. View a cell on edge. Is it perfectly flat? Find a group of cells and notice how they are joined together. Search for small spherical bodies. These are the salivary corpuscles, and are in reality escaped lymphoid cells from the adenoid tissue at the root of the tongue.

Place upon the slide some of the scrapings from the pharynx (upper part) of a frog. Examine with H. P. and observe the elongated cells with cilia in motion. Ciliated cells may also be demonstrated from scrapings of the macerated trachea of a pig or ox.

Examine the scrapings of the stomach of some animal. Observe the columnar cells. Scrape the cut surface of a liver with a scalpel and mix the scrapings with normal saline. Examine and search for hepatic cells, representing glandular epithelium.

Epithelium of a frog. Macerate the larva of a frog or salamander in dilute alcohol, cut the casts from the skin into small pieces, and apply one of these to a slide and stain with hæmatoxylin, method No. 5.

MEMORANDA.

EPITHELIAL TISSUE.

A. Squamous epithelium. B. Columnar epithelium.

A. Squamous epithelium: (a) Squamous cell; (b) Cell wall; (c) Protoplasm; (d) Nucleus; (e) Nucleolus; (f) Cell on edge; (g) Group of cells; (h) Salivary corpuscle; (i) Epithelium of a frog.

B. Columnar epithelium: (a) Columnar cells from stomach showing cell wall and nucleus—flat surface; (b) Columnar cells—end view.

A. Ciliated epithelium. B. Glandular epithelium.

A. Ciliated cells: (a) Cells, showing cell-wall and nucleus; (b) Cilia; (c) Goblet cells; (d) Section of stratified ciliated epithelium.

B. Glandular epithelium: (a) Cuboidal cells from liver; (b) Nucleus.

CHAPTER VIII.

CONNECTIVE TISSUE.

Connective tissue is derived from the mesoblast and consists of cells and intercellular substance. It is found between the skin and mucous membranes. It differs chiefly from epithelial tissue in having a greater amount of intercellular substance. Its functions are to connect different structures and furnish support to the organs of the body. The cells entering into it are of two kinds, fixed and wandering. The fixed cell is a flattened plate with nucleus, protoplasm, and enclosing membrane. Sometimes there are projections of the cell-wall which give a stellate appearance. Wandering cells (such as leucocytes) are those which migrate from place to place in the tissue.

There are ten important kinds of connective tissues—viz., white fibrous tissue, yellow elastic tissue, areolar tissue, adipose tissue, mucous tissue, retiform tissue, basement membranes, cartilage, bone, and dentine.

I. WHITE FIBROUS TISSUE.

This form is composed of delicate fibrils, often collected into bundles. The bundles may run parallel with each other or interlace, forming a mesh-work. This tissue is found in tendons, the omentum, subcutaneous tissues, etc. In a tendon the fibrils compose parallel primary bundles, and these unite to form secondary bundles, each of which is enveloped in a delicate sheath. All are bound together to form the tendon, which is encased in a tough sheath of connective tissue, septa from which extend inward, enclosing the secondary bundles.

Laboratory exercise No. 17.—*Tendon.* Embed a piece of tendon in celloidin. Stain sections with carmine, method No. 2. Make out the external sheath and the septa of connective tissue. Observe the branched spaces for tendon cells. Demonstrate, if possible, the primary bundles and the ends of the fibrils. Label and preserve. Drawings.

II. YELLOW ELASTIC TISSUE.

This consists of highly refracting fibres which form a network and are often associated with the preceding tissue. The fibres, when

free from their attachments, become bent or coiled, and, when boiled, yield elastin, and not gelatin, as is the case with white fibres. This structure occurs in the *ligamentum nuchæ, ligamenta subflava,* walls of bronchioles and alveoli of lungs, arteries, vocal cords, and in connective tissue generally. This and the preceding form of connective tissue are intercellular in character and are, therefore, associated with cells in the formation of tissues. For the practical study of elastic fibres, the *ligamentum nuchæ* of an ox may be used.

III. AREOLAR TISSUE.

Areolar tissue is composed of cells and an intercellular substance which consists chiefly of white and elastic fibers. It lines the under surface of the skin, forms the muscle sheaths, and is found in the mammary gland and other structures.

IV. ADIPOSE TISSUE.

Adipose tissue is almost wholly cellular. The cells are probably formed from connective tissue corpuscles, in which fat globules appear, increase in size, and finally coalesce. Thus is formed one large globule of fat, which distends the cell-wall, crowding the protoplasm and nucleus outward. The cells are well supplied with blood capillaries. They are bound together by areolar tissue into lobules, and the lobules into lobes. For practical study, a section of the tongue will be found satisfactory. It is widely distributed, occurring almost everywhere that connective tissue is found.

V. MUCOUS TISSUE.

This occurs in the umbilical cord and comprises cells and a gelatinous intercellular substance called the *jelly of Wharton.* The cells are stellate in form, and the protoplasmic processes anastomose with each other, forming a mesh-work throughout the structure. This tissue contains but few fibrous elements, except as the cord approaches full time.

Laboratory exercise No. 18.—*Umbilical cord.* Embed pieces of a three-months' umbilical cord in celloidin. Stain with hæmatoxylin, method No. 5. Observe (1) a thin layer of superficial cells; (2) the blood-vessels, two arteries and one vein, surrounded by the mucous tissue, the jelly of Wharton, or mucin, containing the stellate cells; (3) look also for white or elastic fibres. In what other structure is mucous tissue found?

VI. ADENOID TISSUE.

Adenoid tissue consists of a network of fibrils holding in their meshes lymphoid cells and leucocytes. The fibrils are supposed to be derived from cell-processes, which anastomose with each other. The nuclei of the cells appear at the intersections of the fibrils. It occurs in lymphatic glands, tonsils, solitary glands, and Peyer's patches, many mucous membranes, spleen, and thymus gland.

VII. BASEMENT MEMBRANE, OR MEMBRANA PROPRIA.

The basement membrane, or *membrana propria,* consists of a delicate homogeneous membrane, composed of flattened plates of cellular origin, and occurs as a supporting base for the epithelial cells which line mucous membranes and the acini and ducts of glands.

VIII. CARTILAGE.

Cartilage is a dense tissue made up of an enclosing sheath, the *perichondrium,* a somewhat hyaline *matrix,* either homogeneous or fibrous, and *cells.*

The *perichondrium* is the enclosing sheath of connective tissue, and consists of two layers, an outer layer of dense fibrous tissue and an inner, looser layer. The terms fibrous and chondrogenetic apply respectively to these layers. The latter is so called because it is chiefly engaged in the formation of cartilage. It contains cells arranged in parallel rows, which multiply by mitosis.

The *matrix* is a dense, intercellular substance of a white, bluish, or yellowish color. It may be homogeneous or more or less supplied with white or yellow fibres.

Embedded in the matrix are the *cartilage cells.* These cells are usually oval in outline, but are sometimes flattened on one side, the flattened surface being toward the periphery. They are arranged in pairs and groups, and are seldom crowded, except toward the perichondrium. Each cell is enclosed by a *capsule.* The space within the capsule, which is filled up by the cell, is called a *lacuna.* There are three varieties of cartilage, determined by the presence or absence of white and yellow fibres. They are *hyaline cartilage, elastic cartilage,* and *fibro-cartilage.*

(1) HYALINE CARTILAGE.

This is characterized by a bluish-white, semi-transparent matrix, free from fibres. It occurs in costal cartilages, the articular ends of bone, the trachea, bronchi, larynx, the auditory meatus, and in the early cartilage of the fœtus.

Laboratory exercise No. 19.—*Hyaline cartilage.* Harden pieces of costal cartilage, or of the sternum of a newt, in picric acid; embed in celloidin; stain with hæmatoxylin, method No. 5, or carmine, method No. 2. Examine with L. P. and note the following structures: (1) The perichondrium with its fibrous and chondrogenetic layers; (2) the matrix, free from fibres, in which are embedded the cells; (3) the cells, lying in their lacunæ with inclosing capsule, and arranged in groups of two or more. Compare the form, size, and disposition of the more centrally located cells with those toward the surface. Do you observe nucleoli? Drawings.

(2) ELASTIC CARTILAGE.

This form is characterized by the presence of yellow elastic fibres in the matrix. They first appear as minute granules, which arrange themselves in linear rows and, coalescing, form the fibres. The color of the matrix is yellowish. Elastic cartilage occurs in the epiglottis, the external ear, the Eustachian tube, etc.

Laboratory exercise No. 20.—*Elastic cartilage.* Harden portions of the ear of a pig in picric acid, embed in celloidin, and stain with hæmatoxylin, method No. 5. Observe, as in the preceding preparation, the perichondrium, matrix, and cells. Note also the yellow, interlacing fibres, which extend from the matrix into the perichondrium. Minute granules of elastin will also be seen with the high power. Make out the capsule, lacuna, and cell structure. Prepare drawings to illustrate all of these structures.

(3) FIBRO-CARTILAGE.

Here we have the same structures as exhibited in hyaline cartilage, but the matrix is provided with many bundles of white fibres, running in different directions and interlacing. It occurs in the intervertebral disks, sesamoid bones, etc.

Laboratory exercise No. 21.—*Fibro-cartilage.* Decalcify and harden with picric acid pieces of the vertebral column of a cat so as to include the intervertebral disk, or use the intervertebral disk of an ox. Freeze or embed in celloidin. Stain with lithium carmine, method No. 2. Examine and note the numerous bundles of wavy fibres, between which

are the cartilage cells, each inclosed in a thick capsule. Make drawings of all cartilage structures.

IX. BONE.

Bone is a compact, hard form of connective tissue. It comprises two varieties—*spongy* and *compact*. The spongy form occurs in the vertebræ and the ends of the long bones. Compact bone is formed from the spongy variety by the deposit of lamellæ in the intratrabecular spaces. It is found chiefly in long bones between the articular ends. A bone comprises three characteristic structures—viz., the *periosteum*, the *bone-proper*, and the *marrow*.

The **periosteum** consists of two layers, the *fibrous layer* of dense fibrous connective tissue which, as a protecting sheath, covers the outer surface, and the *osteogenetic layer*, a somewhat loose structure, rich in cells and blood-vessels. This layer is so called because it assists in forming bone. Its cells eventually become the *osteoblasts*, which are the bone builders. There are slender portions of the periosteum which project into the bone proper. They are called the *perforating fibers of Sharpey*. They are fibers of the periosteum which have failed to ossify.

The **bone-proper** is composed of cartilage and the carbonate and phosphate of lime. Structurally considered, it comprises the *Haversian systems*, the *inter-Haversian systems*, and the *fundamental lamellæ*. A Haversian system comprises the *Haversian canal*, the *lamellæ*, *lacunæ*, *canaliculi*, and the *bone cells*. The Haversian canal is a minute channel, from 20 μ to 100 μ in diameter, extending longitudinally and opening upon both the inner and outer surface of the bone-proper. It contains an extension of the marrow, and is rich in blood-vessels, cells, and lymphatics.

The lamellæ are plates of bone substance formed in the spaces between the lacunæ and arranged concentrically around the canals. The lacunæ are the cavities which contain the bone cells. The cavities which are excavated by and contain the osteoclasts are called *Howship's lacunæ*. The canaliculi are the slender tubes which radiate from the lacunæ and serve as lymph channels, distributing nutrient fluids throughout the Haversian system. They anastomose with each other and are connected with the canals. The bone cells are the corpuscles within the lacunæ. They send out processes into

the canaliculi. They are derived from the osteoblasts by the incorporation of the latter within the bone matrix. Besides the Haversian system (with its lamellæ) just described, there are the *inter-Haversian,* or *interstitial lamellæ,* and the *fundamental lamellæ.* The fundamental lamellæ cover the free surfaces of the bone adjacent to the periosteum and the marrow. The canals of these lamellæ are styled *Volkman's canals.*

The medulla, or marrow, occupies the central cavity. It is derived from the osteogenetic layer of the periosteum. It is composed of a connective tissue reticulum filled with cells and supplied with an elaborate system of blood-vessels. The connective tissue cells, or marrow cells, in young bone, become the osteoblasts; but, in old bone, deteriorate into fat cells. Primary marrow is red, but in the adult bone it becomes yellow, owing to the formation of fat. The marrow also contains certain large cells which are agents in the destruction of bone. They are called *giant cells, osteoclasts,* or *myeloplaxes.* They multiply by free cell-formation, and are also found in the osteogenetic layer of the periosteum. The marrow is considered an extension of this layer.

Bone formation.—Bone is formed by two methods—centrally, within the cartilage, and superficially, by the periosteum. By the first method a center of ossification is produced by the transformation of the cartilage cells into osteoblasts. By these cells a central core of bone (or bone areas) is formed. At the same time a layer of bone is formed beneath the periosteum, and trabeculæ are thrown out from the osteogenetic layer, which extend to the center of ossification and absorb the endochondral bone, thus producing a central cavity for the marrow. By means of the osteoblasts the permanent bone is now produced between the marrow-cavity and the periosteum. Spongy bone is constituted of periosteum, a meshwork of trabeculæ, and marrow, rich in osteoblasts, etc., filling up the spaces.

Laboratory exercise No. 22.—*Bone.* Harden and decalcify pieces of long bone in picric acid, freeze or embed in paraffin, and stain with picro-carmine or hæmatoxylin, method No. 5. Examine with L. P. and H. P. Make a study of the periosteum, observing the fibrous layer, consisting of dense fibrous tissue, and the osteogenetic layer, consisting of a loose fibrous reticulum rich in cells and blood vessels. Search for

lacunæ in the bone proper and note their arrangement; also find Haversian canals and make out, if you can, Haversian systems. Examine the marrow and demonstrate marrow cells and osteoclasts. Prepared specimens of dry bone should be examined to demonstrate lacunæ and canaliculi. The bone of a fœtus may be prepared for the study of bone development. The preparations need not be preserved unless especially good. What is the average diameter of the canaliculi? Drawings.

X. DENTINE.

Dentine is one of the connective tissues, being derived from the mesoderm. It is commonly known as ivory and is found in the teeth. It differs from bone in composition and structure. Its intimate structure will be given in the chapter on the teeth.

MEMORANDA.

CONNECTIVE TISSUES.

A. White Fibrous Tissue. B. Mucous Tissue.

A. Tendon: (a) Tendon sheath; (b) Septa; (c) Bundles; (d) Fibrils; (e) Branched cell spaces; (f) Longitudinal section.

B. Umbilical cord: (a) Superficial cells from amnion; (b) Arteries; (c) Vein; (d) Jelly of Wharton; (e) Stellate cells: (f) Connective tissue fibres.

Hyaline Cartilage.

Hyaline cartilage: (a) Fibrous layer of perichondrium; (b) Chondrogenetic layer; (c)Matrix; (d) Capsule; (e) Lacuna; (f) Cartilage cell; (g) Group of cells.

CONNECTIVE TISSUES.

A. Elastic Cartilage. **B. Fibro-Cartilage.**

A. Elastic Cartilage (ear): (a) Perichondrium; (b) Matrix; (c) Capsule; (d) Lacuna and cell; (e) Interlacing elastic fibres.

B. Fibro-cartilage: (a) Matrix; (b) Bundles of fibres; (c) Capsule; (d) Cells and osteoblasts.

Bone.

Bone: A. Periosteum: (a) Fibrous layer; (b) Osteogenetic layer; (c) Cells; (d) Fibres of Sharpey.

B. Bone proper: (e) Haversian system; (f) Haversian canal; (g) Lamellae; (h) Lacunae; (i) Canaliculi; (j) Bone cells; (k) Inter-Haversian systems; (l) Fundamental lamellae; (m) Howship's lacunae and osteoclasts.

C. Marrow: (n) Marrow cells; (o) Osteoclasts; (p) Osteoblasts.

CHAPTER IX.

MUSCULAR TISSUE.

Muscle is a tissue derived from the mesoderm. It is composed of cells and a small amount of intercellular substance, and is endowed with the property of contractility.

OUTLINE OF MUSCULAR TISSUE.

1. Kinds.....
   - Smooth—in all involuntary muscle except heart and pharynx.
   - Striated—in heart, œsophagus, and all voluntary muscle.

2. Structure..
   - Smooth muscle..
     - Cells.....
       - Form.
       - Structure.
     - Bundles.
     - Strata.
     - Sheaths..
       - Epimysium.
       - Perimysium.
   - Striated muscle..
     - Fasciculi, composed of fibres.
     - Fibers....
       - Cells.....
         - Sarcolemma.
         - Sarcoplasm.
         - Nuclei.
       - Sarcostyles...
         - Fibrillae.
         - Discs.
           - Dark.
           - Lateral.
           - Intermediate.

3. Functions.

There are two kinds of muscular tissue—(1) *smooth,* or *vegetative;* (2) *striated,* or *animal.*

Smooth muscle is associated with all involuntary muscles except the heart and pharynx, and is found, therefore, in the digestive tract,

the digestive glands, urinary glands, generative organs, respiratory tract, vascular system, lymphatic glands, skin, etc.

Smooth muscle is composed of spindle-shaped cells, each consisting of a cell-wall, protoplasm, and a centrally-located nucleus. The cells overlap by their extremities and are bound together into bundles, each bundle being surrounded by a membrane of areolar connective tissue, called the *perimysium*. The bundles are disposed in layers, or strata, the whole muscle being covered by the *epimysium*, a connective tissue sheath.

Striped muscle.—This is composed of elongated, somewhat cylindrical cells, each consisting of a cell-wall (sarcolemma), protoplasm (sarcoplasm), and a superficially located nucleus. These cells are attached end to end, thus constituting a slender filament, which is the muscle fibre. The *fibre*, therefore, is encased by a delicate, closely-fitting membrane, the *sarcolemma*. It exhibits striations, or alternate bands of light and dark, called *disks*. The nucleus is to be found upon the surface of the cell just beneath the sarcolemma. Each fibre is composed of *sarcostyles* and *sarcoplasm*. Each sarcostyle consists of a group of *ultimate fibrillæ*, which are held together by the surrounding sarcoplasm. Each ultimate fibril is believed to consist of a *prismatic body*, a *dot*, and a delicate *filament*, the dot dividing the filament midway between the prisms. This peculiar structure is supposed to account for the striations of the fibre, the band of prisms giving rise to the *dark disks*, the delicate filaments producing the *light lateral disks*, and the dots forming the *intermidate disks*. It will thus be seen that a voluntary muscle is composed of bundles, the bundles are composed of fibres, the fibres of sarcostyles, the sarcostyles of fibrillæ, and the fibrillæ of peculiar structural elements, sarcoplasm filling up the spaces between these elements. The fibrillæ are the contractile elements. Contraction takes places in a longitudinal direction, not in every direction, as is the case with naked protoplasm.

Enclosing each fibre is a sheath of areolar connective tissue, the *endomysium*. This is not to be confounded with the sarcolemma, which is a part of the fibre. Surrounding each fasciculus, or bundle, is a larger sheath, the *perimysium;* while the sheath enclosing the whole muscle is the *epimysium*.

The striated fibres of the heart differ from the voluntary striated fibres in being devoid of a sarcolemma and in having their nuclei centrally located.

Laboratory exercise No. 23.—*Striated muscle.* Harden pieces of voluntary muscle of salamander or cat in alcohol, embed in paraffin, and stain with carmine, method No. 3. Make two sections, longitudinal and transverse.

L.S. Observe the fibres running parallel with each other. Using the high power, demonstrate the striations of the fibres—dark, lateral, and intermediate. Examine the ends of the torn fibres and search for the ultimate fibrils. Try and demonstrate the sarcolemma. The tissue of a salamander will give the best results.

T.S. Examine a section of an entire muscle, if possible, and demonstrate the epimysium, perimysium, and endomysium. Focus upon the end of a fibre and locate the nucleus near the outer surface. What are Cohnheim's areas?

Incubator.

Bausch & Lomb Optical Co., Rochester, N. Y.

MUSCULAR TISSUE.

Striated Muscle.

Striated muscle: T. S.: (a) Epimysium; (b) Pirimysium; (c) Endomysium; (d) Fasciculus; (e) Fibre; (f) Nucleus; (g) Cohnheim's area.

L. S.: (h) Fibre; (i) Sarcolemma; (j) Dark disk; (k) Lateral disk; (l) Intermediate disk; (m) Nucleus; (n) Sarcostyle; (o) Ultimate fibril.

Smooth Muscle.

Smooth Muscle: (a) Single cells; (b) Nucleus; (c) Group of cells; (d) Perimysium; (e) Epimysium.

CHAPTER X.

NERVOUS TISSUES AND SYSTEMS.

1. NERVOUS TISSUE.

Nervous tissue is derived from the epiderm of the embryo and enters into the structure of the central, sympathetic and terminal systems, and is, therefore, very widely distributed. The following outline exhibits something of the more common characteristics of nervous tissue:

OUTLINE OF NERVOUS TISSUE.

- Cells
  - Structure
    - Nucleus.
    - Protoplasm.
  - Kinds
    - Neuroblast.
    - Neuron.
    - Neuro-dendron.
    - Unipolar.
    - Bipolar.
    - Multipolar.
    - First-type.
    - Second-type.
    - Neuroglia-cell or glia-cell.
  - Processes
    - Axis cylinder.
    - Collaterals.
    - Dendrites.
- Ganglia
  - Cells.
  - Fibres.
  - Sheath.
- Fibres
  - Medullated.
    - Structure
      - Neurilemma.
      - Medullary sheath.
      - Axilemma.
      - Axis cylinder.
      - Fibrils.
      - Nodes of Ranvier.
      - Internodes.
      - Nerve corpuscle.
    - Nerve endings
      - Central.
      - Peripheral.
  - Non-medullated
    - Axis cylinder.
    - Fibrils.
    - Nodes and internodes.
    - Nerve corpuscle and Neurilemma.

Nervous tissue is composed of cells, ganglia, and nerve fibres. **A nerve cell** is devoid of a cell-wall; its nucleus has a prominent nucleolus, and its protoplasm sometimes contains yellowish granules of pigment. The cells are the most abundant in nerve centers—the brain, spinal cord, and ganglia. The primitive nerve cells are called *neuroblasts,* and in the course of their development, by sending out certain processes known as *axis cylinders* and *dendrites,* produce the different forms described as the *dendron, neuro-dendron, unipolar, bipolar,* and *multipolar* cells, and *first-type* and *second-type* cells. An *axis cylinder* is a slender protoplasmic growth from the pointed end of a neuroblast. The *dendrites* are protoplasmic growths which arise from other parts of the cell. The axis cylinder attains considerable length, often as much as a meter; the dendrites are short and slender. The axis cylinder gives off a few lateral processes called collaterals; the dendrites branch dichotomously, forming a dense network. The axis cylinder functions as a nerve fibre; the dendrites possibly serve as a supporting framework for the neuroplasm, and are supposed to be continuous with the ultimate fibrils. A nerve cell with its axis cylinder is a *neuron;* a cell with dendrites and an axis cylinder bearing collaterals is a *neuro-dendron.* Cells are named also from the number of processes they bear—*unipolar,* one process; *bipolar,* two processes; *multipolar,* several processes. A *first-type cell* is one which has a long axis cylinder that becomes a medullated nerve fibre. A cell of the *second-type* has a short axis cylinder which divides and subdivides. The *neuroglia-cells* are small bodies which give off a multitude of fibrils forming a supporting network, serving as connective tissue elements, and holding together the delicate structures which enter into nerve centers. They are called *glia cells,* and the reticulum produced by them is *neuroglia.*

Ganglia are nerve centers consisting of groups of cells and fibres. Some of the fibres which enter the ganglion terminate in its cells, while others pass through to more distant points. The whole ganglion is invested with a connective tissue sheath, and each fibre is enclosed with an endoneurium, which is continuous with the capsule with which the fibre terminates. The brain and spinal cord may be considered as groups of large ganglia.

Fibres.—A nerve fibre is derived from an axis cylinder of a ganglion cell. A ganglion cell is a cell of the first-type, producing a medullated fibre. The sheath which invests the whole fibre is the *neurilemma,* or *primitive sheath.* It is a thin, transparent, tough membrane of areolar connective tissue. Directly beneath the neurilemma is the *medullary sheath,* which consists of a semi-fluid, highly-refracting substance, called *myelin.* Beneath the medullary sheath and immediately surrounding the axis cylinder is a delicate and very thin investment, the *axilemma.* The axis cylinder is the essential part of the fibre. It may be naked, or covered with medullary sheath alone, or with neurilemma alone, or with both. The medullary sheath is absent in the nerves of the sympathetic system and occasionally in those of the cerebro-spinal system. The neurilemma is absent in the fibres which traverse the brain and spinal cord. It is also absent in the fibres of the olfactory nerves. At certain regular intervals, the medullated fibres lose their medullary sheath, and the neurilemma closes down upon the axis cylinder. These points are called the *nodes of Ranvier.* The space between two nodes is the *internode.* About midway of the internode, just beneath the neurilemma, is the *nerve corpuscle.*

The non-medullated fibres occur in the sympathetic system. Each fibre consists of axis cylinder (composed of a bundle of fibrils), the neurilemma, and an oval nucleus upon the surface of the fibre.

II. NERVOUS SYSTEMS.

There are three important nervous systems—central nervous system, sympathetic system, and terminal system.

CENTRAL NERVOUS SYSTEM.

This is sometimes called the cerebro-spinal system. It consists of the spinal cord and brain. Each of these is constituted of ganglion cells, nerve fibres, neuroglia, and connective tissue.

SPINAL CORD.

The spinal cord, located in the spinal column, consists of gray and white nervous matter. The gray matter is in the center of the cord, and the white matter surrounds it. The gray matter is made

up of ganglion cells, neuroglia, and fibres, while the white matter consists chiefly of medullated fibres and connective tissue.

OUTLINE OF THE SPINAL CORD.

- Fissures
  - Anterior median fissure.
  - Posterior median septum.
- White substance
  - Anterior lateral column.
  - Posterior lateral column
    - Column of Goll.
    - Column of Burdoch.
- Gray substance
  - Anterior gray commissure.
  - Posterior gray commissure.
  - Central canal.
  - Lateral columns
    - Anterior cornua
      - Anterior roots.
      - Posterior roots.
    - Posterior cornua.
      - Reticular process.
      - Column of Clark.
      - Substantia gelatinosa.
    - Lateral cornua.
  - Multipolar cells
    - Structure
      - Nucleus and nucleolus.
      - Nuclear membrane.
      - Axis cylinder.
      - Dendrites.
    - Motor cells
      - Antero-median group.
      - Postero-lateral group.
    - Column cells.
- Neuroglia
  - Ependymal cells.
  - Deiter's cells.
- Investments
  - Dura mater.
  - Arachnoid membrane.
  - Pia mater.
  - Septa from pia mater.

The **surface** of the spinal cord comprises four areas: *Anterior, posterior,* and two *lateral areas.* The cord is divided vertically into two lateral halves by the *anterior median fissure* and the *posterior median septum.* These fissures do not extend through the commissures.

Each half of the spinal cord is divided into the *anterior column, posterior column,* and *lateral column.* These divisions are marked by furrows indicating the exit of the anterior and posterior roots of the spinal nerves. In the upper thoracic and lower cervical regions, two divisions appear in the posterior column, a median portion, called the *column of Goll,* and a lateral portion, the *column of Burdoch.*

The **white matter** is held together by septa of connective tissue which proceed from the pia mater. Under the microscope it appears to be made up of a vast number of nerve fibres, exhibiting an axis cylinder, as a central dot, surrounded by a lighter substance, the medullary sheath.

The **gray substance** of the spinal cord is centrally located, and appears in the form of the letter H—i. e., two irregular bands connected by a bridge. The bridge consists of the *anterior gray commissure* and the *posterior gray commissure.* Between the two commissures is the *central canal.* This extends through the cord longitudinally, and is lined with ciliated epithelium. It is about 1mm in diameter. The commissures consist of fibres derived from the commissure cells. The *white commissure* is immediately in front of the anterior gray commissure. Each lateral column of the gray substance consists of three well-marked divisions—the *anterior, posterior,* and *lateral cornua.* From the anterior cornua emerge the *anterior roots,* while the *posterior roots* enter the posterior cornua. The *reticular process, column of Clark,* and the *substantia gelatinosa* enter into the structure of a posterior cornu. The reticular process is a net-like mass of gray substance at the base of the cornu. The column of Clark is on the median side, near the gray commissure. The substantia gelatinosa covers the horn and immediately surrounds the central canal. The *substantia gelatinosa Neurolandi, zona spongiosa,* and *zona terminalis* are also found in the posterior cornu.

In its intimate structure the gray matter consists of multipolar cells, neuroglia, and medullated fibres. The *multipolar cells* are of two kinds—*motor cells* and *column cells.* The motor cell has a large body with long protoplasmic processes, the dendrites, and an axis cylinder, which, emerging from the anterior cornu, becomes invested with a medullary sheath, thus producing the axis cylinder of a nerve fibre. The fibres of the anterior roots originate from the motor cells. The column cells are smaller than those of the motor type and have fewer dendrites. The axis cylinders from these cells largely make up the white substance of the cord. The axis cylinders of column cells send out many collaterals which penetrate the gray matter. After entering the white matter, each cylinder divides into two stem-fibres, ascending and descending, which extend longitudinally through the cord, giving off many collaterals, which return to the gray matter and terminate in tufts of fibrils, the stem-fibres eventually terminating in the same way. The axis cylinders which originate in Clark's column do not divide when they reach the white substance, as do those of the column cells, but proceed upward to the cerebellum.

The supporting framework of the gray matter consists of neuroglia, which is composed of *glia cells* and their processes. There are two kinds of glia cells—the *ependymal cells* and *Deiter's cells.* Ependymal cells are of the epithelial type and line the lumen of the central canal. They are provided with cilia and send out processes into the surrounding tissues. Deiter's cells are found in the gray matter, and afterwards in the white. They send out delicate processes, which form a supporting framework for the delicate nerve structures.

The investments of the spinal cord are the *dura mater,* on the outside; the *arachnoid membrane,* centrally located; and the *pia mater,* immediately surrounding the cord. From the pia mater, septa of connective tissue extend inward.

There are thirty-one pairs of nerves springing from the spinal cord. The motor nerves are anterior, while the sensory nerves are posterior. These nerves are distributed to the muscles, skin, and other parts of the body, where they are provided with special terminations adapted to receive impressions.

BRAIN.

The brain comprises the medulla oblongata, the cerebellum, and the cerebrum. It is directly connected with the spinal cord, and, thus, with the nerve structures of all parts of the body. It sends forth twelve pairs of cranial nerves, which supply the organs of sense, lungs, heart, etc. It possesses two kinds of nervous matter, gray and white. The arrangement of these substances is the reverse of that exhibited in the spinal cord, except that in the medulla oblongata the gray matter is centrally located. The gray matter is found in the cerebral cortex, the corpora striata, optic thalami, corpora quadrigemina, cerebral ganglia, the lining of the ventricles, and the cerebellar cortex.

The whole brain is encased with three characteristic membranes, the dura mater on the outside, next to this the arachnoid membrane, and on the inside, immediately investing the brain, the pia mater.

The dura mater is a dense, elastic, fibrous membrane. It sends three processes into the brain for its protection and support, and also penetrates the skull. The arachnoid membrane lies between the *dura* and *pia,* and is extremely thin and delicate. Between the arachnoid and pia is a space filled with the cerebro-spinal fluid. The membrane is composed of white fibres and elastic tissue. The pia mater consists of a plexus of blood vessels held together by delicate areolar tissue.

OUTLINE OF THE BRAIN.

- Medulla oblongata
  - Fissures
    - Anterior median.
    - Posterior median.
    - Two lateral fissures.
  - Surfaces
    - Anterior.
    - Lateral.
    - Posterior.
  - Substances
    - White matter.
    - Gray matter.
- Cerebellum
  - Cerebellar cortex
    - Molecular layer.
    - Layer of Purkinji's cells.
    - Granular layer.
  - Medulla, or white matter
    - Medullated fibres.
    - Connective tissue.

- Cerebrum
  - Cerebral cortex
    - Granular layer.
    - Layer of small pyramidal cells.
    - Layer of large pyramidal cells.
    - Layer of irregular cells.
    - Layer of fusiform cells.
  - Medulla
    - Medullated fibres.
    - Connective tissue.
  - Ventricles
    - Gray matter
      - Multipolar ganglion cells.
      - Neuroglia.
      - Medullated fibres.
    - White matter
      - Medullated fibres.
      - Connective tissue.
- Brain investments
  - Dura mater.
  - Arachnoid membrane.
  - Pia mater.

MEDULLA OBLONGATA.

The structure of the medulla oblongata is virtually the same as that of the spinal cord. The chief difference consists in the arrangement of the structural elements.

CEREBELLUM.

The cerebellum consists of the cerebellar cortex and medulla.

The *cerebellar cortex* comprises three layers—the *molecular layer, layer of Purkinji's cells,* and *granular layer.* The *molecular layer* is composed of large and small multipolar ganglion cells. The small cells are disposed toward the surface. The large cortical cells are in the deeper portion of the cortex and send out protoplasmic processes toward the surface. *Purkinji's cells* are large, somewhat pear-shaped ganglion cells, which send out two large protoplasmic processes from one pole of each cell into the molecular layer. The axis cylinder proceeds from the opposite pole, becomes a medullated fibre, and enters the white matter of the cerebellum. The *granular layer* is the innermost, and is composed of large and small cells provided with large nuclei. The whole layer presents a rusty appearance. Each cell is provided with dendrites and a non-medullated nerve process, which extends into the molecular layer.

The *white matter* consists of *medullated fibres* and *neuroglia.* There are two kinds of neuroglia cells in the cerebellum: First, *small rounded cells,* which occur in the granule layer and send a few processes downward and many longer processes outward to the molecular layer; second, *stellate cells,* which are found in all the layers.

CEREBRUM.

The cerebrum comprises the cerebral cortex, medulla, and ventricles.

The cerebral cortex is composed of five layers which merge imperceptibly into each other. They are:

(*1*) *Molecular Layer.*—This is superficial, and is finely granular, with an interlacing of medullated fibres and a reticulum of neuroglia. The cells of Caji are found in this layer.

(*2*) *Layer of Small Pyramidal Cells.*—This is composed of ganglion cells which are pyramidal in form. They send out several apical and lateral dendrites, which penetrate the molecular layer and, by continuous division, produce a complicated network. The axis cylinder proceeds from the basal end of the cell and, after sending out a few collaterals, enters the medulla.

(*3*) *Layer of Large Pyramidal Cells.*—This differs from the preceding in the size of its cells. The axis cylinder process enters the medulla to become a medullated fibre.

(*4*) *Layer of Irregular Cells.*—This is composed of cells, oval or polygonal, which are devoid of apical dendrites. Each axis cylinder sends out collaterals and then enters the medulla to become one or two nerve fibres.

(*5*) *The Layer of Fusiform Cells.*—This lies adjacent to the medulla and is composed of a few spindle-shaped cells with nerve fibres between them. The cells are arranged parallel with the course of the fibres.

The medulla.—This comprises the white matter of the cerebrum, and is composed of medullated fibres and connective tissue structures.

The ventricles.—The gray matter of the ventricles, like that of the cerebral cortex, is composed of multipolar ganglion cells, neu-

roglia, and medullated fibres. The cells give rise to axis cylinders which produce the cranial nerves. The white matter of the ventricles consists of medullated fibres and neuroglia.

THE SYMPATHETIC NERVOUS SYSTEM.

The sympathetic system consists of a double chain of ganglia (about twenty-four pairs) extending along the anterior portion of the spinal column and connected together by intervening cords, three great plexuses (cardiac in cervical region, epigastric in abdominal region, and hypogastric in pelvic region), smaller ganglia, and many non-medullated nerve fibres. The nerve fibres are of two kinds—communicating and distributory. They are non-medullated, and by some authorities are supposed to be without a neurilemma.

TERMINAL NERVOUS SYSTEM.

The peculiar modifications of nerve endings may appropriately be considered as constituting a third nervous system, the *terminal system,* often styled the peripheral system.

Every nerve has central and peripheral terminations. The central ending is in the cell with which it originates. There are three important varieties of peripheral endings for sensory nerve fibres—viz., *free nerve endings, terminal corpuscles,* and *neuro-epithelial cells.*

1. **Free nerve endings** occur in the skin, mouth, cornea, and spinal cord. By this method the nerve fibre loses its neurilemma and medullary sheath, and the axis cylinder breaks up into delicate fibrils, which sometimes anastomose with each other. In the skin the fibrils are confined to the stratum Malpighii.

2. **Terminal corpuscles** occur in the skin. Stirling and Piersol give four kinds of peripheral corpuscles—(1) *simple tactile cells,* (2) *compound tactile cells,* (3) *end bulbs,* (4) *touch corpuscles.*

Simple tactile cells occur in the stratum Malpighii. The fibre breaks up into fibrils by the usual method, but each fibril terminates in a tactile disk, above which lies the tactile cell.

In compound tactile cells, the tactile disk forming the termination of the axis cylinder lies between two tactile cells.

End bulbs occur in mucous membranes, the cutis, and other

places. The bulbs vary in shape, round to cylindrical, and consist of a fibrous capsule and a central core in which terminates an axis cylinder. The Pacinian corpuscle, which occurs in the subcutaneous tissue of certain localities, is a variety of the same and is peculiar in exhibiting many concentric fibrous laminæ.

Touch corpuscles occur in the papillæ of the skin. The nerve fibre as it enters the corpuscle breaks up into fibrils, which form a coil, giving a striated appearance.

3. Neuro-epithelium.—This occurs in the perceptive organs and consists of highly specialized cells, such as the rod and cone cells of the retina, and olfactory and gustatory cells. These cells receive the stimuli from external sources, the nerve-fibres conveying them onward to the nerve centers.

Laboratory exercise No. 24.—*The spinal cord.* Harden pieces of the spinal cord of a cat in Muller's fluid, embed in paraffin, and stain with hæmatoxylin and eosin, method No. 8. Examine first with L. P., then with H. P. Study the structure of the inclosing membranes. Observe the septa of connective tissue extending from the pia mater into the underlying tissue. Find the posterior median septum, and the anterior median fissure. How can you always distinguish the posterior from the anterior surface of the cord? Note the difference in appearance between the white matter and gray matter. Make a study of the white matter, noting the axis cylinders with their medullary sheaths. Find the posterior and anterior lateral columns. Make a study of the gray matter, observing the anterior and posterior lateral cornua. Look for the anterior and posterior roots of the spinal nerves. Observe the central commissure inclosing the central canal. Multipolar ganglion cells should be found in the gray matter. Follow out an axis cylinder until it enters the white matter. Look for neuroglia. Drawings.

Laboratory exercise No. 25.—*The cerebrum.* Harden in Muller's fluid, embed in paraffin, and stain with hæmatoxylin and eosin, method No. 8. In examining the cerebrum, search first for the pia mater; then demonstrate the cerebral cortex with its five layers made up of cells of different forms and sizes; and, finally, the medulla, consisting chiefly of medullated fibres. The layers of the cerebral cortex, beginning externally, will be arranged in the following order: Granular layer, layer of small pyramidal cells, layer of large pyramidal cells, layer of polymorphous cells, and the layer of fusiform cells. Demonstrate the axis cylinders and their collaterals; observe the bundles of medullary fibres extending between the cells. Drawings.

Laboratory exercise No. 26.—*The cerebellum.* Harden in Muller's fluid, embed in paraffin, and stain with hæmatoxylin and eosin, method

No. 8. An examination of the cerebellar cortex will exhibit three layers —the molecular layer, the layer of Purkinji's cells, and the granular layer, located internally. The white matter will be found to be made up of medullated fibres and neuroglia. Observe the primary and secondary convolutions. Notice the cells of Purkinji and their protoplasmic processes. Note also the pia mater. Drawings.

NERVOUS TISSUE.

A. Cells. **B. Fibres.**

A. Cells: (a) Neuroblast; (b) Neuron; (c) Neuro-dendron; (d) Unipolar cell; (e) Bipolar cell; (f) Multipolar cell; (g) First-type cell; (h) Second-type cell; (i) Glia cell; (j) Axis cylinder process; (k) Collaterals; (l) Dendrites.

B. Fibre: (a) Neurilemma; (b) Medullary sheath; (c) Axilemma; (d) Axis cylinder; (e) Nodes of Ranvier; (f) Internode: (g) Nerve corpuscle.

C. Ganglion, exhibiting cells and fibres and sheath.

D. Nerve: (a) Fibres; (b) Epineurium; (c) Perineurium; (d) Endoneurium.

Spinal Cord.

Spinal cord: (a) Pia mater; (b) Septa; (c) Anterior median fissure; (d) Posterior median fissure; (e) Anterior lateral column; (f) Posterior lateral column; (g) Column of Goll; (h) Column of Burdoch; (i) Anterior gray commissure; (j) Posterior gray commissure; (k) Central canal; (l) Anterior Cornu; (m) Posterior Cornu; (n) Lateral Cornu; (o) Motor cells; (p) Column cells; (q) Neuroglia.

NERVOUS TISSUE.

Cerebrum.

Cerebrum: (a) Dura mater; (b) Arachnoid; (c) Pia mater; (d) Septa; (e) Cerebral cortex; (f) Medulla; (g) Molecular layer; (h) Layer of small pyramidal cells; (i) Large pyramidal cells; (j) Layer of irregular cells; (k) Layer of fusiform cells; (l) Medullated fibres; (m) Axis cylinder; (n) Neuroglia.

Cerebellum.

Cerebellum: (a) Pia mater; (b) Cerebellar cortex; (c) Molecular layer; (d) Layer of Purkinji's cells; (e) Granular layer; (f) Medulla; (g) Medullated fibres; (h) Neuroglia; (i) Primary convolutions; (j) Secondary convolutions; (k) Purkinji's cells and processes.

CHAPTER XI.

THE CIRCULATORY SYSTEM.

The circulatory system includes the heart, arteries, veins, and capillaries. It is derived from the mesoderm of the embryo. Its nervous supply is received from the sympathetic system. The following outline exhibits the structural elements which enter into its various organs:

OUTLINE OF CIRCULATORY SYSTEM.

- Heart
  - Layers
    - Pericardium
      - Visceral endothelium.
      - Fibrous tissue.
      - Areolar tissue.
      - Parietal endothelium.
    - Muscular layer, or myocardium
      - Striated fibres.
    - Endocardium
      - Elastic fibres.
      - Substantia propria.
      - Endothelium.
  - Annuli fibrosi.
  - Cavities
    - Auricles.
    - Ventricles.
  - Valves
    - Fibrous connective tissue.
    - Endocardium.
- Arteries
  - Tunica adventia
    - Fibrous connective tissue.
    - Elastic fibres.
  - Tunica media
    - Involuntary muscle.
    - Elastic tissue.
  - Tunica intima
    - Internal elastic membrane.
    - Sub-endothelial tissue.
    - Endothelium.
- Veins
  - Tunica adventia
    - Connective tissue.
    - Elastic fibres.
    - Smooth muscle.
  - Tunica media
    - Involuntary muscle.
    - Elastic tissue.
  - Tunica intima
    - Internal elastic membrane.
    - Sub-endothelial tissue.
    - Endothelium.
    - Valves.

Capillaries .... { Endothelial cells. Cement. Stigmata.

THE HEART.

The heart is composed of three distinct layers—*pericardium; muscular layer,* or *myocardium;* and *endocardium.*

The **pericardium** is lined on its outer and inner surfaces with a single layer of endothelium. Between these layers are fibrous and areolar tissues, which send out septa into the myocardium.

The **muscular layer,** or *myocardium,* is composed of striated fibres which branch, and the branches anastamose with each other. The fibres run in all directions, interweaving with each other, thus making possible the peculiar contraction of the heart. The fibre is without a sarcolemma and has centrally-located nuclei.

The **endocardium, or inner layer,** is composed of *elastic fibres,* the *substance proper,* and *endothelium.* The substance proper contains smooth muscle fibres, which are surrounded by a delicate perimysium.

The **annuli fibrosi** consist of ligaments which lie between the auricles and ventricles, and form an attachment for numerous muscle fibres.

The *valves* of the heart are modified endocardium. They consist of endocardium and fibrous connective tissue which is continuous with that of the annuli fibrosi.

THE ARTERIES.

Arteries comprise three coats—*Tunica adventitia, tunica media,* and *tunica intima.* The *tunica adventitia* is the external layer and consists of bundles of connective tissue and elastic fibres, longitudinally disposed. The *tunica media* is composed of involuntary muscle and elastic tissue, circularly disposed. The *tunica intima* consists of the internal elastic membrane, which is structureless in character; the subendothelial tissue, which consists of flattened corpuscles and elastic fibres; and the endothelium, consisting of a single layer of cells lining the internal cavity of the artery.

VEINS.

A vein also consists of three coats similarly named to those of the artery. A vein differs from an artery in being thinner, and in having a preponderance of connective over muscular and elastic tissues.

The *tunica adventitia* is composed of bundles of connective tissue, elastic fibres, and involuntary muscle, which intercross, the general arrangement being longitudinal. In the *tunica media,* the smooth muscle fibres are circularly arranged and are associated with fibro-elastic tissue. The *tunica intima* comprises the internal elastic membrane, the subendothelial tissue, and a single layer of polyhedral endothelial cells.

The valves of the veins are produced by an infolding of the intima. Their surfaces are lined with endothelial cells.

CAPILLARIES.

A capillary consists of a single layer of endothelial cells united to each other by a cement substance and exhibits at intervals open spaces, the *stigmata.* It is through the stigmata that the leucocytes migrate on their mission to build up the worn-out tissues of the body. Capillaries which supply nutrition to blood-vessels are called *vasa vasorum.* The diameter of a capillary averages about 8 μ.

Laboratory exercise No. 27.—*The heart.* Fix and harden with alcohols, embed in paraffin, and stain with hæmatoxylin, method No. 6. In examining your preparation, search first for the pericardium, and demonstrate its endothelial lining and fibrous connective tissue; then examine the myocardium, observing the course of the fibres, their branching and anastomosing, and the centrally located nuclei; finally, examine the endocardium, composed of fibrous tissue, elastic fibres, and endothelium. If possible, examine the structure of a valve. Search for blood-vessels. Drawings.

Laboratory exercise No. 28.—*Arteries and veins.* Harden the great aorta in alcohol, embed in paraffin, and stain with hæmatoxylin and eosin, method No. 8. Examine with H. P. and make out the three coats —the tunica adventitia on the outside, tunica media in the middle, and tunica intima on the inside. Name the structures which enter into these coats. A study of veins may be made from sections of the umbilical cord, the lungs, or the tongue. Drawings.

CIRCULATORY SYSTEM.

Heart.

Heart: (a) Pericardium, exhibiting endothelial layers and intervening fibrous tissue; (b) Myocardium: (c) Endocardium, with its elastic fibres, substantia propria and endothelium; (d) Annuli fibrosi; (e) Anastomosing striated fibres; (f) Nucleus; (g) Valve.

A. Artery. **B. Capillary and Vein.**

A. Artery: (a) Tunica adventitia; (b) Tunica media: (c) Tunica intima.
B. Vein: (a) Tunica adventia; (b) Tunica media; (c) Tunica intima; (d) Capillary.

CHAPTER XII.

THE LYMPHATIC SYSTEM.

The lymphatic system includes lymphatic tissues, vessels, organs. Lymphatic tissue is immediately concerned in the production of lymph corpuscles. The yellowish fluid contained in the lymphatic vessels is the lymph. The structures of this system are derived from the epiderm and mesoderm.

OUTLINE OF THE LYMPHATIC SYSTEM.

- **Lymphatic tissue**
  - Adenoid tissue, or connective tissue reticulum.
  - Lymphoid cells.
- **Lymphatic vessels**
  - Outer coat.
  - Middle coat.
  - Inner coat.
- **Lymphatic follicles**
  - Simple nodules
    - Capsule.
    - Adenoid tissue and lymphoid cells.
  - Compound lymphatic follicles
    - Capsule and trabeculæ.
    - Cortex
      - Cortical follicles.
      - Germinal center.
    - Medulla.
    - Hilum.
    - Sinuses.
    - Reticulum.
- **Spleen**
  - Capsule
    - Serous coat.
    - Fibrous coat.
    - Trabeculæ.
  - Splenic pulp
    - Divisions
      - Loose adenoid tissue.
      - Dense adenoid tissue, or Malpighian corpuscles.
    - Structure
      - Connective tissue reticulum.
      - Cells
        - Leucocytes.
        - Red corpuscles.
        - Lymphoid cells.
        - Pigment granules.
- **Thymus body**
  - Derivation
  - Capsule.
  - Lobes
    - Septa.
    - Lobules
      - Cortex.
      - Medulla.
      - Hassal's corpuscles.

Tonsils ..... { Capsule. Adenoid tissue. Mucous glands. Blood and lymph corpuscles.

LYMPHATIC TISSUE.

Lymphatic tissue is composed chiefly of two structures: (1) **A connective tissue reticulum.** This is commonly known as adenoid, or retiform tissue. (2) **The lymphoid cells,** which are held in the meshes of the reticulum. These escape into the lymphatic vessels and are there known as the lymph corpuscles. They eventually become the leucocytes of the blood.

LYMPHATIC VESSELS.

The lymphatic vessels are irregular in outline, due to folds in the endothelium, which serve as imperfect valves. The wall of each vessel exhibits three coats. The inner coat consists of endothelium, the middle of smooth muscle, and the outer of connective tissue.

LYMPHATIC FOLLICLES.

These have been classified as simple nodules and compound lymphatic nodes. The **simple nodule** consists of a *capsule* and *adenoid tissue* with *enclosed lymphoid cells.* The **compound lymphatic node** exhibits *capsule, reticulum of connective tissue, cortex,* and *medulla.* The *capsule* is the sheath of connective tissue which covers the node and from which septa, or trabeculæ, extend into its substance. The *cortex* is the outer zone of the body, and consists of the cortical follicles and the germinal center. The *cortical follicles* are the laboratories in which are manufactured the leucocytes. The *germinal center* is a light spot within the follicle, characterized by the presence of kinetic figures. The *medulla* is the central portion of the gland. It contains the medullary bands, which are simply extensions of the trabeculæ into its substance. This mesh-work formed by the subdivision of the trabeculæ constitutes the reticulum. The *hilum* is an infolding of the capsule upon a blood-vessel or lymph channel at its point of entrance into the gland. *Sinuses* are the open spaces between the adenoid tissue and trabeculæ.

THE SPLEEN.

The spleen has the structure of a large lymphatic node. It consists of a capsule and splenic pulp. The **capsule** consists of two coats—an outer serous and inner fibrous coat. Extending from the capsule into the pulp are *trabeculæ*, which divide and subdivide, forming a reticulum of connective tissue. The **splenic pulp** comprises the *loose adenoid tissue* and the *dense adenoid tissue.* The dense adenoid tissue is disposed in spherical masses which are situated in the forks of the arteries. Each mass is pierced by an artery. These dense spherical masses of the pulp are called *Malpighian corpuscles.* Their structure does not differ materially from that of the loose tissue. The pulp, as with other lymphatic structures, consists of a connective tissue reticulum, holding in its meshes leucocytes, red corpuscles, lymphoid cells, and pigment granules.

THYMUS BODY.

This structure is derived from the hypoderm, and in its early development is chiefly epithelial in character, but later becomes invaded with mesodermic tissues, until it assumes a lymphatic type. After the second year it loses its characteristic structure, and its tissues are replaced by fibrous tissue and fat. It consists of a capsule and a pulp, which is divided into lobes. The lobes are separated from each other by septa derived from the capsule. The lobes are divided into lobules, each lobule consisting of adenoid tissue, the outer, looser portion being called the cortex, while the inner, denser portion is the medulla. *Hassal's corpuscles* are masses of embryonic epithelial cells found in the medulla.

TONSILS.

The tonsils are composed of diffuse adenoid tissue, containing from ten to eighteen lymph follicles. There is a fibrous capsule, beneath which are the connective tissue reticulum, the lymphatic follicles, mucous glands between the follicles, and lymphoid cells.

Laboratory exercise No. 29.—*The spleen.* Harden in Erlicki's fluid or alcohol, embed in paraffin, and stain with hæmatoxylin and eosin, method No. 8. Examine, first, the capsule, and its extensions, or trabeculæ, penetrating the splenic pulp. Distinguish between the loose and dense adenoid tissue. Examine the structure of a Malpighian corpuscle with H. P., noting the connective tissue reticulum and the lymphoid cells. Describe the disposition of arteries, veins, and lymphatics in the spleen. Has the spleen a duct? Drawings.

LYMPHATIC SYSTEM.

Lymphatic Follicle.

Compound lymphatic node: (a) Capsule; (b) Cortex; (c) Cortical follicle; (d) Germinal center; (e) Mudulla; (f) Hilum; (g) Sinus; (h) Reticulum; (i) Lymphoid cells.

Spleen.

Spleen: (a) Capsule—serous coat; (b) Fibrous coat; (c) Trabecula; (d) Loose adenoid tissue; (e) Malpighian corpuscle; (f) Reticulum; (g) Lymphoid cells; (h) Leucocytes; (i) Blood vessels.

CHAPTER XIII.

MEMBRANES AND GLANDS.

I. MEMBRANES.

Membranes are disposed upon surfaces in the interior of the body.

OUTLINE OF MEMBRANES.

- Serous membranes
  - Serous membranes proper.
  - Synovial membranes.
  - Endothelium.
- Mucous membranes
  - Epithelium.
  - Basement membrane.
  - Stroma
    - Fibrous connective tissue.
    - Elastic fibers.
  - Muscularis mucosæ.
  - Occasional structures
    - Papillæ.
    - Glands.
    - Lymphoid cells.

There are two kinds of membranes—serous and mucous. **Serous membranes** cover the outside surfaces of the alimentary tract and respiratory organs, as well as the articular ends of bones and other structures. They include three varieties—serous membranes proper, synovial membranes, and endothelium. Serous membranes proper are composed of fibrous tissue and elastic fibres, with a superficial layer of endothelium. Synovial membranes include the capsules which envelop the ends of joints and the sheaths in which tendons glide. They are characterized by the viscid fluid—the synovia—which they secrete. Endothelium consists of a single layer of flattened endothelial cells.

Mucous membranes line all surfaces which are directly or indirectly in communication with the external atmosphere. The structure of the mucous membrane exhibits the following elements: (1) *The epithelium.* This may be *simple* or *stratified.* It may be squamous, columnar, or ciliated. Columnar and ciliated epithelia

are usually provided with goblet cells, whose function is to produce mucus. With squamous stratified epithelium, the mucus is usually produced by glands located in the stroma. (2) *Basement membrane.* This occurs as a mere line beneath the epithelial layer. It is sometimes called the membrana propria. It is made up of flattened cells, either united edge to edge, or held together by anastamosing processes. On one side, this membrane supports the epithelium; while on the other, it is in close connection with the capillaries. (3) *The stroma.* This structure lies directly beneath the basement membrane, and is composed of fibrous connective tissue and elastic fibres, and is provided with a rich supply of blood-vessels and lymphatics. (4) *The Muscularis mucosæ.* This is a shallow, muscular sheath, which forms the lower limit of the mucous membrane. It is composed of circular and longitudinal strata of involuntary muscle fibres. (5) *Occasional structures.* In addition to the structures above named, papillæ, villi, glands, and lymphoid cells occur in the mucous membrane. *Papillæ* are conical elevations of the stroma, which are supplied with blood-vessels and nerves, and covered with epithelium. They occur in the tongue, œsophagus, etc. The *villi* occur chiefly in the small intestine. They are provided with blood capillaries and lacteals, and are designed to increase the absorbing surface of the intestine. The *glands* which occur are of two varieties—tubular and sacular. The mucous glands are located in the epithelial layer and stroma of mucous membranes, and are concerned in the elaboration of mucin.

II. GLANDS.

Glands are of two kinds—serous and mucous. A *serous* gland is characterized by spherical, granular cells, with central nuclei, which are readily stained by carmine. *Mucous* glands are not readily stained by carmine. The nuclei are usually upon the cell surface.

OUTLINE OF GLANDS.

Kinds..... { Serous glands. / Mucous glands.

- Forms
  - Tubular
    - Simple.
    - Compound.
  - Saccular
    - Simple.
    - Compound, or racemose.
- Structure
  - Fundus
    - Basement membrane.
    - Secretory cells.
  - Lumen.
  - Duct.
- Development.

There are two characteristic forms of glands—tubular and saccular—either of which may be simple or compound. A *simple tubular gland* is cylindrical and elongated, consisting of a basement membrane lined internally with cuboidal or columnar cells which constitute the *fundus,* the opening from the gland being styled the *duct.* The empty space within the gland is the *lumen.* When the lumen becomes divided by one or more partitions, producing a cluster of simple glands, we then have a *compound tubular gland.* The *saccular gland* resembles in structure that of the tubular variety, but in form it is broader, becoming inflated like a sac. A group of simple saccular glands gives rise to the *compound saccular,* or *racemose,* variety. The chief function of glands is that of secretion. The salivary glands, pancreas, and intestinal glands are illustrations.

III. PAROTID GLAND.

The Parotid gland is a compound saccular gland of the serous type. Its tissues are derived from the mesoderm and epiderm. Its function is to produce saliva, a watery substance containing minute granules.

OUTLINE OF THE PAROTID GLAND.

- Capsule.
- Septa.
- Lobes
  - Lobules
    - Acini
      - Secretory cells.
      - Basement membrane.
      - Lumen.
    - Ductules.
  - Connective tissue.
  - Ducts
    - Stenson's duct.
    - Salivary tubes.

Structure.—There is an investing *capsule* of fibrous connective tissue which sends out *septa* into the substance of the gland; these septa divide the gland into *lobes* and *lobules*. Each *lobule* is composed of *acini*, each *acinus* consisting of a basement membrane lined with secreting cells. The lumen of the acinus, or alveolus, leads to the *alveolar ductule*, the ductules discharging into the *salivary tubes;* the salivary tubes into the *intermediate tubes;* and these into *Stenson's duct*, which is the large excretory duct. Stenson's duct is composed of fibro-elastic tissue lined internally with a single layer of columnar epithelium.

Laboratory exercise No. 30.—*Parotid gland.* Harden in alcohol, embed in paraffin, and stain with carmine, method No. 3. It is well in demonstrating most structures to begin with the external, simpler parts, and work inward to those that are more complex. In this case demonstrate first the capsule and its penetrating septa. Note how the gland is divided into lobes and lobules by the subdividing septa. Observe an acinus, and note the form of the cell. Find Stenson's duct and note its structure. Drawings.

IV. THE PANCREAS.

The pancreas is a compound tubular gland of the serous type. The following outline exhibits its structure:

OUTLINE OF THE PANCREAS.

Capsule.

Septa.

Lobes ..... { Acini. Gland cells. Bodies of Langerhans.

Pancreatic Duct.

Intermediate tubules.

Structure.—The pancreas has the usual investing *capsule* of connective tissue, from which extend numerous *septa* dividing the organ into *lobes*. The lobes consist of *acini*, whose basement membranes are lined with short cylindrical or conical cells. Each cell is characterized by two zones—a clear peripheral zone containing the nucleus, and the zone next the lumen which contains the so-called *zymogen granules*. The *bodies of Langerhans* are imper-

fect acini which appear as less dense rounded areas among the ordinary tissue. For the elimination of the products of secretion, the pancreas is provided with *ductules, intermediate tubules,* and the *pancreatic duct.* The last is composed of fibrous connective tissue lined internally with epithelium and provided with minute mucous glands.

Laboratory exercise No. 31.—*Pancreas.* Harden with alcohol, embed in paraffin, and stain with carmine. Make out the following structures: The capsule, septa, lobes, acini, basement membrane, secretory cells, containing nuclei and granules, intermediate tubules, and pancreatic duct. Drawings.

MEMORANDA.

GLANDS.

Forms of Glands.

A. Simple tubular gland: (a) Basement membrane; (b) Secretory cells; (c) Lumen; (d) Duct.

B. Compound tubular gland: (a) Basement membrane; (b) Cells; (c) Ductule; (d) Duct.

C. Simple saccular gland.

D. Compound saccular gland.

A. Parotid Gland. **B. Pancreas.**

A. Parotid gland: (a) Capsule; (b) Septa; (c) Lobe; (d) Lobule; (e) Acinus; (f) Secretory cells; (g) Basement membrane; (h) Lumen; (i) Ductule; (j) Stenson's duct; (k) Connective tissue.

B. Pancreas: (a) Capsule; (b) Septa; (c) Lobe; (d) Acinus; (e) Gland cells; (f) Bodies of Langerhans; (g) Pancreatic duct; (h) Intermediate tubules.

CHAPTER XIV.

THE SKIN.

The epidermis of the skin is derived from the epiderm of the embryo, while the corium is largely of mesodermic origin. Its chief functions are protection, respiration, and excretion.

OUTLINE OF SKIN.

- Derivation.
- Structure
  - Epidermis
    - Stratum corneum.
    - Stratum lucidum.
    - Stratum granulosum.
      - Pigment granules.
    - Stratum malpighii....
      - Prickle cells.
  - Corium....
    - Fibrous connective tissue.
    - Elastic fibres.
    - Blood vessels, nerves, etc.
- Appendages
  - Glands....
    - Sebaceous.
    - Sudoriferous.
  - Nails.....
    - Body.....
      - Free edge.
      - Nail wall.
    - Root.....
      - Nail fold.
    - Nail bed.
    - Matrix.
  - Hair.....
    - Shaft.....
      - Cuticle.
      - Cortex.
      - Medulla.
    - Root.....
      - Bulb.
      - Papilla.
      - Follicle.

Structure.—The skin consists of two layers—the *epidermis,* or *cuticle,* and *corium,* or *cutis.* The **epidermis** is constituted of stratified squamous epithelium disposed in four layers. These are the *stratum corneum, stratum lucidum, stratum granulosum,* and *stratum Malpighii.* The *stratum corneum* is superficial and is composed of flattened corneous cells which have lost their protoplasm and, by desquamation, are continually cast off. The *stratum lucidum,* occurs next, and comprises a narrow zone in which the cells exhibit an approach to the flattened scales of the outer stratum.

The *stratum granulosum* contains cells more flattened than those of the succeeding layer, and possesses granular particles, called *eleidin,* which have an affinity for carmine. In the *stratum Malpighii,* the cells are rounded, or even columnar, in shape. Some of the cells are connected by protoplasmic processes, which give rise to the so-called prickle cells. In this layer occur the pigment granules that give color to the skin. Beneath the epidermis is a *basement membrane.* The **corium** comprises all that part of the skin underlying the epidermis. It consists of two layers—the *stratum papillare* and the *stratum reticulare.* The stratum papillare contains the *papillæ,* which are conical elevations of the corium into the epidermis. They are supplied with blood-vessels and nerves, a nerve-ending occurring at the summit of each papilla. The corium is composed of fibrous connective tissue, elastic fibres, blood-vessels, nerves, glands, and a small amount of muscular tissue. The sebaceous glands are located in the corium papillare, while the sudoriferous glands occur in the corium reticulare or subcutaneous tissue. The corium rests upon a subcutaneous structure, which consists chiefly of areolar, adipose, and fibrous connective tissues.

APPENDAGES.

The **nails.**—Nails are derived from the epidermis, and are composed of horny cells. The structure of the nail comprises the body, root, nail-bed, and matrix. The *body,* or exposed part, terminates in a free-edge, while its sides are protected by the nail-walls. The groove which receives the root is the nail-fold. That portion of the epidermis upon which the nail rests is the *nail-bed,* and the basal portion of the nail-bed upon which the root rests is the *matrix.* The matrix and nail-bed are constituted of the stratum Malpighii, while the body and root represent the stratum lucidum, the stratum corneum being absent. The portion which is actively engaged in producing the nail-body is the matrix. The nail, therefore, grows in length, thickness, and width by the formation of new cells by the matrix. The body consists of horny plates.

The **hair.**—Hair is a modification of the epidermis, and is produced by an infolding of the epidermis and a differentiation of the lower cells of the follicle thus produced. The hair consists of two

parts, the shaft and the root. The *shaft* comprises the *cuticle,* or outer, imbricated layer of epithelial cells; the *cortex,* or middle layer, composed of elongated, horny, epithelial cells, which contain the pigment granules that give color to the hair; and the *medulla,* which constitutes the central cylinder and is composed of cuboidal cells filled with minute air vesicles which appear as dark granules. These air vesicles also contribute to the color of the hair. The *follicle* is the receptacle of the hair, produced by the infolding of the epidermis. Above the sebaceous glands it consists of a fibrous coat, stratum lucidum, and stratum corneum, but beneath them the stratum corneum disappears. The root and bulb of the hair exhibit a continuation of the same essential structures as found in the shaft. The *papilla* is a conical elevation of the corium into the base of the follicle and hair bulb. It contains pigment cells and blood-vessels, which provide nourishment.

A transverse section of a hair varies in outline with different races. The hair of the Mongolian (Japanese) exhibits a circular outline; that of a German is oval; that of a Negro is flattened-ovate; while that of the Papuan is still more flattened and crescent-shaped. The hair is provided with *sebaceous glands,* which are of the simple and compound saccular type. These open into the hair follicles near their upper extremity. The *sebum,* or secretion, which they discharge upon the surface of the hair is designed to oil it and keep it in a healthy condition. Each hair is also provided with a muscle, the *arrector pili* (composed of smooth muscle fibre), which extends obliquely from the deeper portion of the follicle to the upper portion of the corium.

Sebaceus glands.—These are of the simple or compound saccular type, and are located in the stratum papillare of the corium. They consist of acini, with secreting cells, and a short duct.

The **sudoriferous glands.**—The sweat glands are of the simple tubular variety. The coil of the gland is located in the subcutaneous tissue, and consists of a basement membrane lined with secreting cells. The excretory duct extends through the corium and epidermis by a sinuous course until it reaches the stratum corneum, when it becomes spiral, terminating upon the surface of the skin in a rounded pit.

Laboratory exercise No. 32.—*The skin.* Harden portions of the scalp and palm of the hand in alcohol, embed in paraffin, and stain with hæmatoxylin and eosin, method No. 8. Make a study of the structures described above, noting especially the stratum corneum, stratum lucidum, stratum granulosum, and stratum Malpighii of the epidermis. Observe the membrana propria which forms a supporting base for the squamous cells. Note the difference in form of the cells of the different layers. In the corium, observe the conical elevations, or papillæ, and the hair follicles which extend into its structures. The corium is made up chiefly of connective tissue filled with blood-vessels and nerves. In the subcutaneous tissue, search for sweat glands and trace the excretory duct to the surface. Make a study of the hair, both T.S. and L.S., and demonstrate the shaft, root, bulb, papilla, hair follicle, cuticle, cortex, and medulla. Drawings.

MEMORANDA.

THE SKIN AND ITS APPENDAGES.

The Skin.

Skin: (a) Epidermis; (b) Stratum corneum; (c) Stratum lucidum; (d) Stratum granulosum; (e) Stratum malpighii: (f) Prickle cells; (g) Corium; (h) Fibres of connective tissue; (i) Blood vessels; (j) Nerve endings; (k) Papilla; (l) Sweat gland; (m) Duct of same; (n) Subcutaneous tissue.

Hair.

Hair: (a) Follicle: (b) Shaft; (c) Cuticle; (d) Cortex; (e) Medulla; (f) Root; (g) Bulb; (h) Papilla; (i) Sebaceous gland; (j) Hair muscle; (k) Hair of German; (l) Hair of Negro; (m) Hair of Japanese; (n) Hair of Papuan.

CHAPTER XV.

THE ALIMENTARY CANAL.

The alimentary canal is derived from the hypoderm and mesoderm. The squamous epithelium which lines the œsophagus is of epidermic origin. The columnar epithelium which lines the alimentary tract from the cardiac orifice onward is derived from the hypoderm. The connective tissue and muscles of the tract are of mesodermic origin. A careful study of the subjoined outlines will indicate to the student the very close analogy between the different structures of the canal. The chief differences will be found in the pharynx and œsophagus. The upper half of the pharynx is lined with ciliated epithelium, its lower half and the whole course of the œsophagus being provided with stratified squamous epithelium. The stomach and intestine have, instead, columnar epithelium. The pharynx and œsophagus are provided with striated muscle fibre, whereas the stomach and intestine have only smooth muscle. The four coats which are present in all these structures are the mucosa, sub-mucosa, muscular coat, and serous, or fibrous, coat. The mouth is lined with stratified squamous epithelium, beneath which is the tunica propria, or connective tissue stroma, which consists of interlacing bundles of fibrous connective tissue containing elastic fibres. There are numerous papillæ in the tunica propria, and a large supply of small mucous racemose glands.

ALIMENTARY CANAL.

- Œsophagus....
  - Mucosa.....
    - Squamous epithelium.
    - Membrana propria.
    - Stroma.
    - Muscularis mucosæ.
  - Sub-mucosa ....
    - Fibrous connective tissue.
    - Elastic fibres.
    - Glands.
  - Muscular coat.....
    - Circular muscle layer.
    - Longitudinal muscle layer.
  - Serous coat.....
    - Fibrous connective tissue.
    - Elastic fibres.

- **Stomach**
  - Mucosa
    - Columnar epithelium.
    - Membrana propria.
    - Stroma.
    - Peptic and pyloric glands.
    - Muscularis mucosæ.
  - Sub-mucosa
    - Connective tissue.
    - Elastic bundles.
    - Glands.
    - Vessels.
  - Muscular coat
    - Circular muscle layer.
    - Longitudinal muscle layer.
  - Serous coat
    - Connective tissue.
    - Endothelium.

- **Small intestine**
  - Mucosa
    - Columnar epithelium.
    - Basement membrane.
    - Stroma.
    - Capillaries and lacteals.
    - Muscularis mucosæ.
    - Villi.
    - Glands of Lieberkuhn.
  - Sub-mucosa
    - Connective tissue.
    - Vessel.
    - Glands.
      - Brunner's glands.
      - Solitary glands.
      - Peyers' patches.
  - Muscular coat
    - Circular layer.
    - Longitudinal layer.
  - Serous coat.

- **Large intestine**
  - Mucosa
    - Columnar Epithelium.
    - Basement membrame.
    - Glands of Lieberkuhn.
    - Stroma.
    - Muscularis mucosæ.
    - Vessels.
  - Sub-mucosa
    - Solitary glands.
    - Peyers' patches.
    - Connective tissue.
  - Muscular coat
    - Circular layer.
    - Longitudinal layer.
  - Serous coat.

THE ŒSOPHAGUS.

The œsophagus contains four characteristic coats—the *mucosa,* the *sub-mucosa,* the *muscular coat,* and the *fibrous coat.*

The **mucosa** consists of stratified *squamous epithelium* resting upon a *tunica propria,* or *connective tissue stroma,* beneath which is a thin layer of involuntary muscle, the *muscularis mucosœ.* The tunica propria is composed of fibrous connective tissue and contains blood-vessels and lymphatics.

The **sub-mucosa** lies beneath the muscularis mucosæ, and consists of loose connective tissue, containing blood-vessels, nerves, and glands of the racemose variety.

The muscular coat, in the upper end of the œsophagus, is composed of striated fibres; in the lower end, of smooth muscle; while the middle portion contains both smooth and striated fibres.

The *fibrous coat* envelops the muscular coat, contains elastic tissue, and forms an attachment to the adjacent areolar tissue.

Laboratory exercise No. 33.—*The Œsophagus.* Harden in corrosive sublimate, embed in celloidin, and stain with carmine, method No. 3. Examine with L. P. Demonstrate the epithelial layer, stroma and muscularis mucosæ of the mucosa. Search for blood-vessels, nerves, and glands in the sub-mucosa. Examine the muscular coat, and determine from your preparation the part of the œsophagus from which the tissue was obtained. Note the structure of the fibrous coat. Is there an endothelial layer? Do you find any investing areolar tissue?

THE STOMACH.

The stomach has the usual coats—mucosa, sub-mucosa, muscular coat, and serous coat.

The **mucosa** is lined superficially with a single layer of *columnar epithelium,* the squamous epithelium of the œsophagus having ended abruptly at the cardiac orifice. Besides the epithelial layer the mucosa contains the *basement membrane,* the *stroma,* the *muscularis mucosœ,* and the *gastric glands.* The gastric glands are of two kinds —*peptic* and *pyloric.* The peptic glands are of the simple tubular variety, consisting of basement membrane, secreting cells, and duct. The mouth of the duct is marked by a slight depression upon the surface of the mucous membrane. These glands are found on the cardiac and middle thirds of the stomach, and are provided with

acid cells. The pyloric glands are distributed upon the pyloric third of the stomach and are of the compound tubular variety. They are not provided with acid cells. They have a wide duct which leads to the narrow lumens of the tubular branches. The stroma consists of connective tissue supplied with capillaries and lymphatics. The *muscularis mucosæ* consists of a double layer of smooth muscle, an inner circular, and an outer longitudinal.

The **sub-mucosa** is composed of loosely-woven bundles of elastic tissue, containing blood-vessels and nerves. Upon its outer surface there are alternate elevations and depressions. These give rise to the so-called *rugæ* and *depressions* of the stomach wall.

The **muscular coat** consists of an inner circular layer and an outer longitudinal stratum of smooth muscle. At the cardiac end there is also a middle oblique layer.

The **serous coat** consists of fibrous tissue and elastic fibres, lined superficially with a single layer of endothelium.

Laboratory exercise No. 34.—*The stomach.* Fix in corrosive sublimate solution, embed in celloidin, and stain with hæmatoxylin and eosin, method No. 8. Use H. P. and L. P. First demonstrate the four coats; then observe the internal lining of columnar cells with their basement membrane. Locate and study the peptic and pyloric glands. Observe the stroma and muscularis mucosæ; also distinguish between the circular and longitudinal muscle layers. How does the serous coat differ in appearance from the sub-mucosa? Drawings.

THE SMALL INTESTINE.

The small intestine comprises the duodenum, jejunum, and ileum. These parts differ but slightly in general structure. There are the four characteristic coats—mucosa, sub-mucosa, muscular coat, and serous coat.

The **mucosa** consists of the following structures: (1) A single layer of *columnar epithelial cells,* which cover the surfaces of conical elevations which stud the intestine, the *villi;* (2) the *basement membrane,* which supports the epithelium; (3) the *stroma,* or *tunica propria,* which forms conical projections, the villi. It consists chiefly of connective tissue. Each villus is supplied, superficially, with a capillary network, and through its center extends a lacteal. (4) The *muscularis mucosæ* consists of a longitudinal layer with occasional circular fibres of smooth muscle.

The **sub-mucosa** consists chiefly of fibro-elastic bundles and penetrating blood-vessels and nerves.

The **muscular coat** is in two layers, an inner circular and an outer longitudinal, separated by connective tissue.

The **serous coat** consists of connective tissue and endothelium.

There are four varieties of glands which occur in the intestinal wall. The *glands of Lieberkuhn* occur in the mucosa and are distributed along the whole course of the small and large intestine, being found between the villi. They are simple tubular depressions provided with basement membranes and secreting cells. The *glands of Brunner* are of the same type as the pyloric glands of the stomach, but owing to repeated division they have more the appearance of the compound sacular than of the tubular variety. They are serous, and not of the mucous type. They occur in the duodenum. The *solitary glands* are to be found in the sub-mucous coat and consist of isolated lymph follicles. They occur in the small and large intestines. *Peyer's patches* are compound glands of the racemose variety. They occur in the mucosa and sub-mucosa. They should be sought in the small intestine, more especially in the ileum. Goblet cells are of frequent occurrence in the stomach and intestine. The nerve supply of the alimentary tract is from the cranial and sympathetic nerves.

Laboratory exercise No. 35.—*Small intestine.* Fix in corrosive sublimate, embed in celloidin, and stain with hæmatoxylin and eosin. Examine with L. P. and H. P., and demonstrate the following structures: Columnar epithelium, goblet cells, membrana propria, villi, tunica propria, capillaries, glands of Lieberkuhn, Brunner's glands, Peyer's patches, solitary glands, muscularis mucosæ, sub-mucosa, circular muscle layer, longitudinal muscle layer, serous coat, and endothelium. An injected specimen should be examined to demonstrate the capillary network. Drawings.

LARGE INTESTINE.

The large intestine differs chiefly from the small intestine in possessing thicker walls and fewer glands. It is supplied with *solitary follicles* and the *glands of Lieberkuhn,* the latter containing many goblet cells. There are the usual coats—*mucosa, sub-mucosa, muscular coat,* and *serous coat.*

Laboratory exercise No. 36.—*Large intestine.* Fix in corrosive sublimate, embed in celloidin or paraffin, and stain with hæmatoxylin and eosin, method No. 8. Search for the lacteal in the center of a villus. Demonstrate the different coats and name their structural elements. Find the glands of Lieberkuhn and solitary follicles. Drawings. State the functions of the various glands of the alimentary tract. How are capillaries and lacteals distributed? What of the nerve supply and nerve endings?

Œsophagus.

Œsophagus: (a) Mucosa; (b) Squamous epithelium; (c) Membrana propria; (d) Stroma; (e) Muscularis mucosæ; (f) Sub-mucosa; (g) Connective tissue; (h) Glands; (i) Circular muscle layer; (j) Longitudinal muscle layer; (k) Serous coat; (l) Endothelium.

Stomach.

Stomach: (a) Mucosa; (b) Columnar epithelium; (c) Membrana propria; (d) Stroma; (e) Peptic glands; (f) Pyloric glands; (g) Muscularis mucosæ; (h) Submucosa; (i) Connective tissue; (j) Blood vessels; (k) Nerve; (l) Circular muscle layer; (m) Longitudinal muscle layer; (n) Serous coat.

ALIMENTARY TRACT.

Small Intestine.

Small Intestine: (a) Mucosa; (b) Columnar epithelium; (c) Basement membrane; (d) Stroma; (e) Muscularis mucosæ; (f) Villus; (g) Capillaries; (h) Lacteal; (i) Gland of Lieberkuhn; (j) Sub-mucosa: (k) Connective tissue; (l) Brunner's gland; (m) Solitary gland; (n) Peyer's patch; (o) Circular muscle layer; (p) Longitudinal muscle layer; (q) Serous coat.

Large Intestine.

Large Intestine: (a) Mucosa; (b) Columnar epithelium; (c) Basement membrane; (d) Glands of Lieberkuhn; (e) Stroma; (f) Muscularis mucosæ; (g) Vessels; (h) Sub-mucosa; (i) Solitary gland; (j) Peyer's Patch; (k) Connective tissue; (l) Circular muscle layer; (m) Longitudinal muscle layer; (n) Serous coat.

CHAPTER XVI.

THE LIVER.

The liver is derived from the epiderm and mesoderm. It is developed as a compound tubular gland, but afterwards loses that type. Its functions are secretion and excretion.

OUTLINE OF THE LIVER.

Investment.

Lobes.

Lobules
- Capsule of Glisson.
- Intralobular vein.
- Interlobular veins, arteries, and bile ducts.
- Hepatic cells.
- Bile capillaries.
- Blood capillaries.

Gall cyst
- Mucous membrane.
- Muscular coat.
- Fibrous coat.

The liver is invested with a *capsule* of fibrous connective tissue, which sends prolongations into the substance of the organ, producing a framework for the support of the vessels and cells. The organ is composed of *lobes,* and these are subdivided into *lobules.* Each lobule is surrounded by a sheath of connective tissue, called the *capsule of Glisson.* This capsule contains the interlobular veins (branches of the portal vein), which send capillaries into the substance of the lobule. These converge in the center of the lobule and form the *intralobular vein,* and this unites with others to form sublobular veins, and these, in turn, form the hepatic vein, which conveys the blood from the liver. Between the capillaries are to be found the *hepatic cells,* devoid of cell membranes and containing granular protoplasm and one or two nuclei. The bile-capillaries are between the liver cells and distinct from the blood-capillaries. They are continuous with the interlobular bile-ducts, the latter forming larger ducts which are lined with columnar epithelium. The lobule also contains a small amount of *areolar tissue.* In the cap-

sule of Glisson, therefore, are to be found the *interlobular veins, arteries* (from the hepatic artery), and *bile-ducts.* A thorough knowledge of the liver lobule gives the key to a knowledge of the whole organ.

The **gall cyst,** which receives the contents of the hepatic bile-duct, is composed of *mucous, muscular,* and *fibrous* coats The larger bile-ducts have a fibrous adventitia and a mucous membrane which, with that of the gall cyst, is lined with columnar epithelium.

Laboratory exercise No. 37.—*The liver.* Harden in alcohol, embed in paraffin, and stain with hæmatoxylin and eosin. Observe upon the surface the fibrous investment and note the prolongations of its structure into the interior. Focus upon a single lobule and note the following parts: The interlobular vein, centrally located; the network of radiating blood capillaries, which is easily demonstrated in injected specimens; the bile capillaries between the hepatic cells, which receive the bile elaborated within the lobule and convey it to the larger bile ducts; the hepatic cells, noting their form and arrangement and granular appearance; the capsule of Glisson, containing bile ducts and branches of the portal vein and hepatic artery. Search for bile ducts and the gall cyst, noting their structure. Drawings.

MEMORANDA.

THE LIVER.

Liver Structures.

Liver: (a) Capsule; (b) Lobe; (c) Lobules; (d) Capsule of Glisson; (e) Blood vessels; (f) Hepatic cells.

A. Liver Lobule. **B. Gall Cyst.**

A. Lobule: (a) Capsule of Glisson; (b) Interlobular vein; (c) Interlobular arteries; (d) Interlobular bile duct; (e) Capillary network; (f) Bile capillaries; (g) Hepatic Cells; (h) Intralobular vein.

B. Gall Cyst: (a) Mucous coat; (b) Columnar cells; (c) Muscular coat; (d) Fibrous coat.

CHAPTER XVII.

TONGUE AND TEETH.

I. TONGUE.

The tongue is derived from the epiderm and mesoderm. It consists chiefly of a mucous membrane and bundles of muscle fibers associated with connective tissue. It is the organ of taste, this sense being derived especially from the taste-buds which are located in the squamous epithelium which lines its surface.

OUTLINE OF THE TONGUE.

- **Mucous coat**
  - Squamous epithelium.
  - Membrana propria.
  - Papillæ
    - Filiform.
    - Fungiform.
    - Circumvallate.
  - Taste buds.
  - Furrows and ridges.
- **Muscular tissue**
  - Fibres
    - Branched.
    - Striated.
  - Bundles
    - Vertical.
    - Transverse.
    - Longitudinal.
  - Septum lingualæ.
  - Interfascicular spaces containing
    - Connective tissue.
    - Fat.
    - Glands.
- **Adenoid tissue**
  - Reticulum.
  - Lymphoid cells.

Blood vessels, lymphatics, and nerves.

The tongue is constituted of a mucous coat, muscular tissue, adenoid tissue, blood-vessels, lymphatics, and nerves. The **mucous coat** contains a superficial layer of *stratified squamous epithelium.* Within this layer are located the *taste-buds,* conical bodies which are the seat of the sense of taste and are found upon the fungiform and circumvallate papillæ, and in the epithelium of the dorsum and sides of the tongue. Beneath the squamous epithelial layer is the *mem-*

brana propria. The elevations of the stroma of the mucosa give rise to the papillæ. These are of three forms, *filiform,* or narrow; *fungiform,* or club-shaped; and *circumvallate,* or broad and flat. The circumvallate papillæ are confined to the posterior part of the dorsum. The fungiform varieties occur upon the dorsum. Often secondary papillæ are present.

Glands occur in the stroma and are of the mucous and serous types. The mucous glands are of the compound tubular variety, and occur at the base and edges of the tongue. The serous glands are of the saccular form, and occur near the circumvallate papillæ and taste-buds.

The **muscular tissue** of the tongue consists of striated muscle fibres arranged in bundles which run vertically, transversally, and longitudinally. The spaces between the bundles are filled up with connective tissue, fat-cells, and glands. There is a connective tissue partition, called the *septum lingualæ,* which passes vertically through the middle of the tongue, dividing the muscular tissue into two portions.

The **adenoid tissue** at the base of the tongue is found in the crypts, or depressions, of the mucosa, and consists of a connective tissue reticulum and lymphoid cells. The tongue is richly supplied with blood-vessels, lymphatics, and nerves.

Laboratory exercise No. 38.—*The tongue.* Harden with alcohol, embed in paraffin, and stain with carmine, method No. 3. Study first the mucosa, demonstrating the epithelial layer, basement membrane, tunica propria, and the filiform, fungiform, and circumvallate papillæ. Make a search for taste buds. Observe the loose fibrous structure of the tunica propria and demonstrate, if possible, mucous and serous glands. Notice the disposition of the bundles of striated fibres. Focus upon a fibre with H. P. and demonstrate its striations. In the inter-fascicular spaces observe connective tissue and fat-cells. A section prepared from the base of the tongue should exhibit adenoid tissue in the crypts of the mucosa. Does your section show the *septum lingualae?* Drawings.

II. THE TEETH.

The teeth are derived from the epiderm and mesoderm. The most important structure of the tooth is the *pericementum,* or *peridental membrane,* for upon its healthy action the condition and usefulness

of the tooth depend. It serves to hold the tooth in place, as well as to manufacture the cementum. The cavity which contains the tooth and is lined by the pericementum is called the *alveolus*. The layer produced by the pericementum and covering the root of the tooth is the *cementum*. The corresponding layer upon the crown of the tooth is the *enamel*. The enamel and cementum enclose the *dentine*, commonly called ivory. In the cavity of the tooth, which is surrounded by the dentine, is the *pulp*. The blood and nerve supply of the tooth is conveyed through an opening at the extremity of each root. A single nerve fibre enters each tooth and its branches follow the course of the blood-vessels.

The enamel is derived from the epiderm; the dentine and cementum from the mesoderm. A tooth is developed from the dental ridge, which forms the enamel organ, and from the dental papilla, which consists of mesodermic connective tissue. The latter grows up toward the enamel organ, so that the two are in contact. The enamel organ produces the enamel, and the dental papilla produces the dentine.

OUTLINE OF THE TEETH.

- **Alveolus.**
- **Pericementum.**
  - Fibers.
  - Fibroblasts.
  - Cementoblasts.
  - Osteoblasts.
  - Osteoclasts.
  - Glands.
- **Dentine.....**
  - Matrix.....
    - Fibrous reticulum.
    - Calcareous salts.
  - Dentinal tubules.....
    - Dentinal sheaths.
    - Dentinal fibers.
  - Interglobular spaces.
  - Schreger's lines.
  - Lines of Salter.
- **Enamel.....**
  - Membrane of Nasmyth.
  - Enamel prisms.
  - Stripes of Retzius.
- **Cementum.....**
  - Lamellæ.
  - Lacunæ.
  - Canaliculi.
  - Corpuscles.

Pulp . . . . . { Connective tissue matrix. / Stellate and spindle cells. / Odontoblasts. / Blood vessels and nerves.

Pericementum.—The structural elements of this membrane are the fibres, the fibroblasts, the cementoblasts, the osteoblasts, osteoclasts, and glands. The *fibres* are of the white fibrous variety, and their chief function is to hold the tooth in position. The *fibroblasts* are spindle-shaped cells which occur between the fibres. The *osteoblasts* are the bone formers, and are exactly like those of the periosteum. The *osteoclasts* are the giant cells which have the power to dissolve calcareous matter. *Cementoblasts* engage in the formation of the cementum.

The **cementum.**—This covers the root of the tooth and differs from bone in having no Haversian canals. It is thin at the neck, but becomes thicker toward the extremity of the root. It is made up of lamellæ, lacunæ, cement corpuscles, and canaliculi. The canaliculi are supposed to communicate with the dentinal tubules.

Enamel.—The enamel exhibits the membrane of Nasmyth, the enamel prisms, and the stripes of Retzius. The *membrane of Nasmyth* is a tough epithelial sheath which covers the crown during its earliest development. The *enamel prisms* are five or six-sided rods, which extend out perpendicularly from the dentine. When enamel is attacked by acids, it is completely dissolved, which is not true of dentine and cementum.

Dentine.—Some of the structures of dentine are the dentinal tubules, interglobular spaces, lines of Salter, etc. The *dentinal tubules* are minute canals, which extend from the pulp to the outer surface of the dentine. Each tubule consists of a sheath called the *sheath of Neumann,* within which is a dentinal fibril, a prolongation from an odontoblast of the pulp. The *lines of Salter* are lines which appear in dried specimens, and are probably due to a shrinkage of the tooth. The dentine is composed of twenty-eight per cent of organic matter and seventy-two per cent of inorganic matter. The *interglobular spaces* consist of uncalcified portions of the matrix. They appear as irregular spaces and are sometimes quite abundant.

The **pulp** —This fills the cavity of the tooth within the dentine. It consists of a connective tissue matrix, stellate and spindle cells, round cells, and odontoblasts. The outer surface of the pulp is covered with a layer of *odontoblasts*. Most of the cells beneath the odontoblasts are *stellate* and *spindle-shaped*. The protoplasmic processes of the odontoblasts, which extend into the dentinal tubules, are called the *fibres of Tomes*. The odontoblasts are directly concerned in the manufacture of dentine.

Laboratory exercise No. 39.—*The teeth.* Decalcify in dilute nitric acid, harden in alcohol, embed in celloidin, and stain with hæmatoxylin. Examine a longitudinal section and demonstrate as far as possible the cementum with its lamellæ, lacunæ, cement corpuscles, and canaliculi; also the dentine, exhibiting dentinal tubules, sheath of Neumann, dentinal fibrils, and inter-globular spaces; also the enamel, showing the enamel prisms, matrix, and membrane of Nasmyth. Drawings. The demonstrations of enamel must be made from dry sections, as acids completely dissolve this structure.

MEMORANDA.

TONGUE AND TEETH.

Tongue.

Tongue: (a) Mucous coat; (b) Squamous epithelium; (c) Taste buds; (d) Membrana propria; (e) Filiform papilla; (f) Fungiform papilla; (g) Circumvallate papilla; (h) Submucous tissue; (i) Muscular tissue; (j) Vertical fibers; (k) Transverse fibers; (l) Longitudinal fibers; (m) Septum lingualæ; (n) Interfascicular connective tissue; (o) Fat cells; (p) Adenoid tissue.

Teeth.

Teeth: (a) Pericementum—showing fibers, cementoblasts, etc.; (b) Cementum—showing lamella, lacunæ, canaliculi, and corpuscles; (c) Enamel—exhibiting enamel prisms; (d) Dentine; (e) Dentinal tubule—showing dentinal sheath and fibers; (f) Interglobular spaces; (g) Pulp; (h) Odontoblasts; (i) Stellate cells; (j) Spindle cells.

CHAPTER XVIII.

THE RESPIRATORY SYSTEM.

The respiratory system includes the air passages of the nose, mouth and pharynx, and the epiglottis, larynx, trachea, bronchi, lungs, and pleura. Its structures are derived wholly from the epiderm and mesoderm. Its nerve supply is from the cerebro-spinal and sympathetic systems.

Epiglottis.—This structure consists chiefly of yellow elastic cartilage. Its mucosa possesses many taste-buds and leucocytes.

Larynx.—The thyroid, cricoid, and arytenoid cartilages make up the bulk of the larynx. These are united by fibrous tissue and ligamentous membranes and are covered internally with a mucosa, and externally with fibrous tissue.

THE TRACHEA.

The trachea comprises a mucosa, a sub-mucosa, and fibrous coat. It is largely a mesodermic structure.

OUTLINE OF THE TRACHEA.

- **Mucosa**
  - Ciliated epithelium.
  - Membrana propria.
  - Tunica propria
    - Inner layer.
    - Outer layer.
- **Sub-mucosa**
  - Connective tissue.
  - Glands.
  - Blood vessels, lymphatics, and nerves.
  - Smooth muscle.
- **Fibrous coat**
  - Hyaline cartilage
    - Perichondrium.
    - Matrix.
    - Cells.
  - Connective tissue.

The **mucosa** presents a superficial layer of stratified ciliated epithelium supported by a basement membrane and a tunica propria which possesses a large amount of elastic tissue and is disposed in two layers: An inner layer of loose fibrous tissue containing elastic fibres, nerve fibres, etc., and an outer layer, consisting of a dense network of longitudinal elastic fibres. Of the epithelium, only the

outer layer of cells possesses cilia. Among these occur the goblet cells.

The **sub-mucosa** is made up of loose connective tissue containing glands, blood-vessels, lymphatics, and nerves.

The **fibrous coat** invests the outer surface of the trachea and has embedded in its tissue the incomplete rings of hyaline cartilage. These rings in transverse section appear fusiform in outline, extend over three-fourths, or less, of the circumference, overlap by their edges, and exhibit perichondrium, matrix, lacunæ, and cells. Attached to the perichondrium upon the inner surfaces of the cartilage are transverse bundles of smooth muscle, while across the intervals between the rings are other bundles, the whole contrivance serving to contract the tube. There are but few longitudinal muscular bundles.

Laboratory exercise No. 40.—*Trachea.* Fix with chromic acid, harden with alcohol, embed in celloidin, and stain with hæmatoxylin, method No. 7. Make out the structures of the mucosa, observing especially the layer of ciliated epithelium containing a few goblet cells, the membrana propria, and the two layers of the tunica propria. Search for glands, blood-vessels, etc., in the sub-mucosa. Study the form and structure of the hyaline cartilage in the fibrous layer. Note the disposition of the bundles of smooth muscle. Demonstrate the loose areolar tissue which unites the fibrous coat to adjacent structures. Drawings.

The **bronchi.**—These do not differ materially in structure from the trachea. As the bronchial tubes decrease in size there are certain modifications in structure, such as: (1) The epithelium becomes reduced until in the smallest tubes there is but a single layer of ciliated cells. (2) The elastic tissue disappears from the mucosa and is replaced by a smooth muscle layer that corresponds to the muscularis mucosæ. (3) The cartilage gradually decreases and totally disappears in the terminal bronchioles.

THE LUNGS.

The lungs are derived chiefly from the mesoderm. They resemble in structure racemose glands. Their nerve supply is received from the central and sympathetic systems.

OUTLINE OF THE LUNGS.

- Ducts
  - Bronchial tubes.
  - Bronchioles.
  - Alveolar ducts.
- Spaces
  - Infundibula.
  - Alveoli.
- Pulmonary parenchyma
  - Lobes
    - Lobules
      - Infundibular septa.
      - Alveolar walls.
    - Interlobular tissue.
  - Blood vessels, lymphatics, and nerves.
- Pleura
  - Endothelium.
  - Connective tissue.
  - Subpleural tissue.

The lungs are invested with a connective tissue sheath, the *pleura,* which is made up of endothelium, a connective tissue matrix, consisting of fibrous tissue bundles and elastic fibres, and the subpleural tissue, composed of areolar tissue and elastic fibres. Each lung is comprised of lobes; each lobe, of lobules; and each lobule consists of ducts, air spaces, and pulmonary parenchyma.

The smaller bronchial tubes, or bronchioles, become the terminal bronchioles when their diameter does not exceed 1 mm. They end in the alveolar ducts, which are lined with alveoli, or air sacs; and extending from these ducts are irregular cavities, the infundibula, which are also studded with alveoli.

The pulmonary parenchyma comprises the walls of blood-vessels, lymphatics, bronchioles, and alveolar ducts, the alveolar walls, and the infundibular septa. A bronchiole may be distinguished from a blood-vessel under the microscope by the crenated appearance of its inner surface. The terminal bronchioles are lined with a single layer of ciliated epithelial cells, and their walls consist of elastic fibres and smooth muscle. Each alveolar duct is lined with cuboidal cells, and its wall otherwise resembles that of a bronchiole, but is much thinner. The wall of an alveolus is lined with simple squamous epithelium and comprises also a connective tissue framework and a dense capillary network. The connective tissue frame-

work of elastic fibres surrounds each air sac and forms the septum between adjoining alveoli.

Laboratory exercise No. 41.—*Lungs.* Fix in chromic acid, dehydrate with alcohol, embed in celloidin, and stain with lithium carmine, method No. 4. Make a careful study of the structures above named. Demonstrate arteries, veins, and bronchioles. Distinguish between an alveolus and an infundibulum. With H. P., make out the structure of different portions of the pulmonary parenchyma. Drawings.

Trachea.

Trachea: (a) Mucous coat; (b) Ciliated epithelium; (c) Membrana propria; (d) Tunica propria; (e) Submucous tissue; (f) Hyaline cartilage—showing perichondrium, matrix, and cells; (g) Muscular coat; (h) Fibrous coat; (i) Serous coat.

Lungs.

Lungs: (a) Bronchiole; (b) Alveolar duct; (c) Alveolus; (d) Infundibulum; (e) Infundibular septa; (f) Alveolar wall; (g) Artery; (h) Vein; (i) Pleura.

CHAPTER XIX.

THE URINARY TRACT.

The organs of the urinary tract are the kidney, ureters, bladder, and urethra. Their tissues are derived from the epiderm and mesoderm. The nervous supply is obtained from the cerebro-spinal and sympathetic systems.

THE KIDNEY.

The kidney is one of the parenchymatous organs of the body, illustrating a compound tubular gland and functioning as an organ of excretion. The urine is secreted from the blood in the Malpighian bodies and uriniferous tubules, and contains the products of destructive metabolism.

OUTLINE OF THE KIDNEY.

- **Cortex ...**
  - Capsule.
  - Muscular coat.
  - Labyrinth..
    - Malpighian body .....
      - Capsule of Bowman.
      - Glomerulus.
    - Urineriferous tubule .....
      - Neck.
      - Lumen.
      - Proximal convoluted tubule.
      - Spiral tubule.
      - Descending limb of Henle's loop.
      - Henle's loop.
      - Ascending limb of Henle's loop.
      - Irregular tubule.
      - Distal convoluted tubule.
      - Arched collecting tubule.
      - Straight collecting tubule.
      - Excretory duct, or tube of Bellini.
  - Column of Bertini.
  - Medullary rays.
  - Blood vessels.
  - Stroma.
- **Medulla..**
  - Malpighian pyramids.
    - Base.
    - Papilla.
  - Tubules.
- **Sinus ....**
  - Hilum.
  - Pelvis.
  - Calices.

The outside investment of the kidney is a *capsule* of fibrous tissue, within which is a thin layer of *smooth muscle*. The *cortex* of

the kidney occupies the outer one-third and contains the labyrinth, medullary rays, and blood-vessels. The *labyrinth* comprises the Malpighian bodies and uriniferous tubules. The *Malpighian body* consists of the capsule of Bowman and the glomerulus. The *capsule* is lined with a single layer of flattened epithelial cells. The *glomerulus* consists of a coil of capillaries. Entering each capsule is an afferent artery, and passing from it an efferent vein. *The uriniferous tubule,* which proceeds from the pole of the capsule opposite the entrance of the artery, comprises the following parts: (1) The neck, lined with low cuboidal cells; (2) the convoluted tubule, which is lined with low columnar cells; (3) the spiral tubule, similar in structure to the convoluted portion; (4) the descending limb of Henle's loop, lined with simple squamous epithelium; (5) Henle's loop, composed of polyhedral cells with flattened nuclei; (6) the ascending limb of Henle's loop, with structure resembling that of the loop; (7) the irregular tubule, composed of striated epithelium; (8) the distal convoluted tubule, consisting of granular epithelium; (9) the arched collecting tubule, lined with low cuboidal cells; (10) the straight collecting tubule, which possesses columnar cells; (11) the excretory duct, or tube of Bellini, which is lined with tall columnar epithelial cells.

The *columns of Bertini* constitute the masses of the kidney between the Malpighian pyramids; they extend to the pelvis.

The *medullary rays* are tapering bundles of straight tubules, which extend from the medulla into the cortex.

The *blood-vessels* of the kidney enter at the hilum. The renal artery passes through the sinus, gives off twigs, which extend through the columns of Bertini to the cortex, and enters the Malpighian body by the afferent artery, which splits up into capillaries to form the glomerulus; these unite to form the efferent vein, which unites with similar veins to form the interlobular vein.

The *stroma* constitutes the connective tissue structures, which invest the blood-vessels and tubules.

The **medulla** is composed of from eight to twenty Malpighian pyramids. Each pyramid is made up of tubules, its base corresponding with the line of juncture between the medulla and cortex; and its

apex rests upon the sinus, and by its encroachment upon this structure produces a papilla. The base of the pyramid is capped by the cortical arch.

The **sinus** is the cavity at the basal portion of the kidney. The opening into this cavity is the hilum. The cavity itself is formed by a union of the excretory ducts, thus producing one large lumen, and is invested with a coat which is continuous with the capsule of the kidney, and within this is the wall, which is continuous with that of the ureter. The space within this capsule is the pelvis.

THE URETER.

This structure is composed of three coats—mucous, muscular, and fibrous. The mucous membrane is lined with the so-called transitional epithelium, which consists of few layers, the deeper layers being columnar, and those next the lumen, squamous.

THE BLADDER.

The bladder has three coats—mucous, muscular, and fibrous. The mucous coat is lined with stratified transitional epithelium, the cells presenting considerable irregularity in shape, and often possesssing more than one nucleus.

THE URETHRA.

The urethra consists of mucous and muscular coats. The mucous coat of the female urethra is lined throughout with stratified squamous epithelium. In the male urethra the prostatic portion is lined with transitional epithelium, the intermediate part with stratified columnar cells, while these are succeeded in the penile portion by simple columnar cells.

Laboratory exercise No. 42.—*The kidney.* Harden with alcohol, embed in paraffin, and stain with hæmatoxylin and eosin. Upon the outer surface of your section observe the capsule, and beneath this a delicate layer of smooth muscle. Distinguish between the cortex and medulla. Observe the Malpighian pyramids of the medulla. Their apices form the papillæ, and their bases extend toward the periphery. What elements enter into their structure? The spherical, deeply-stained masses in the medulla are the Malpighian bodies. Demonstrate the capsule of Bowman and glomerulus; also the structure of a tubule, noting its basement membrane, epithelial cells, and lumen. Make a study of other structures enumerated in the outline of this organ. Drawings.

KIDNEY.

Cortex and Medulla.

Kidney: **A. Cortex:** (a) Capsule; (b) Muscular layer; (c) Labyrinth; (d) Malpighian body; (e) Capsule of Bowman; (f) Glomerulus; (g) Uriniferous tubule; (h) Column of Bertini; (i) Medullary ray; (j) Blood vessels; (k) Stroma.

B. Medulla: (l) Malpighian pyramid; (m) Base; (n) Apex, or papilla; (o) Tubules; (p) Sinus—exhibiting hilum, pelvis, and calices.

Uriniferous Tubule.

Uriniferous Tubule: (a) Neck; (b) Lumen; (c) Proximal convoluted tubule; (d) Spiral tubule; (e) Descending limb of Henle's loop; (f) Henle's loop; (g) Ascending limb of Henle's loop; (h) Irregular tubule; (i) Distal convoluted tubule; (j) Arched collecting tubule; (k) Straight collecting tubule; (l) Excretory duct, or tube of Bellini.

CHAPTER XX

THE GENITAL ORGANS.

The reproductive organs are derived from the epiderm and mesoderm. As among many plants, they exhibit sexual characteristics. The function of the male organs is the production of semen, which contains the spermatozoa, or male elements. The female organs produce the ova, female elements, as well as contribute to the development of the fertilized ova and the resulting embryos.

I. MALE REPRODUCTIVE ORGANS.

The male reproductive organs comprise the testis, prostate gland, Cowper's glands, and penis.

THE TESTIS.

The testis is a compound tubular gland whose function is to produce the spermatazoa. It is enveloped in a fibrous capsule and is divided into lobules.

OUTLINE OF THE TESTIS.

- **Tunics**
  - Tunica vaginalis.
  - Tunica albuginea.
  - Tunica vasculosa.
- **Mediastinum, or corpus Highmori.**
- **Septa.**
- **Lobules**
  - Convoluted tubule
    - Parietal cells.
    - Mother cells.
    - Spermatoblasts.
    - Spermatozoa.
  - Tubili recti.
  - Rete testis.
- **Vessels**
  - Vasa efferentia.
  - Coni vasculosi.
  - Tube of epididymus.
  - Vas deferens.
  - Vas aberrans.

The testis is suspended in a sac, the scrotum, and is immediately invested with **three coats**—the *tunica vaginalis,* or serous coat, derived from the peritoneum; the *tunica albuginea,* or dense fibrous coat, which on the posterior border of the testis forms by an inver-

sion of the capsule a much thickened mass, the mediastinum, or corpus Highmori; and the *tunica vasculosa,* containing a plexus of blood-vessels invested by delicate areolar tissue. The septa, which extend from the mediastinum to the tunica albuginea on the opposite side, thus dividing the testis into lobules, are continuous with tunica vasculosa. Each *lobule* is composed of tubules of three kinds —convoluted tubules, tubuli recti, and those of the rete testis. The *convoluted tubule* consists of a basement membrane lined with the sustentacular cells, or parietal layer. Just within this layer are the mother cells, which grow into large cells and multiply by mitosis. Then follow the daughter cells, or spermatoblasts. Within these are to be found the spermatozoa. These tubules are supported by connective tissue derived from the septa, are provided with blind extremities, and each has a membrana propria. The *tubuli recti* comprise the straight tubules formed by the union of the convoluted tubules near the apex of the pyramidal lobule, and possess a basement membrane lined with columnar cells. The *rete testis* consists of anastomosing tubules forming a network within the mediastinum.

The **vessels,** or ducts, associated with the tubules are the vasa efferentia, coni vasculosi, epididymis, vas deferens, and vas aberrans. The tubules of the rete testis emerge from the mediastinum in fifteen or twenty ducts, the *vasa efferentia.* These soon become convoluted in their course, forming conical masses, the *coni vasculosi,* which together constitute the head of the epididymis, or globus major. The *tube of the epididymis* is formed by the union of the ducts of the coni vasculosi. This tube is greatly convoluted, and when unraveled measures upward of twenty feet. The *vas aberrans* is a narrow tube, which extends from the lower part of the tube of the epididymis and is closed by a blind extremity. The *vas deferens* is continuous with the epididymis, and is the excretory duct of the testis. It consists of three coats—epithelial, muscular, and mucous.

THE PROSTATE GLAND.—This is composed largely of smooth muscle, within which are a number of tubular glands, whose ducts open into the urethra. The fibrous capsule is thin, but firm.

COWPER'S GLANDS.—These are two in number, and in

structure are of the compound tubular variety. The excretory duct from each opens into the urethra.

THE PENIS.—The two corpora cavernosa and the corpus spongiosum enter into the structure of this organ. The corpus cavernosum consists of a fibrous sheath, trabeculæ of connective tissue, and smooth muscle. The corpus spongiosum resembles in structure the corpus cavernosum, but its connective tissue is more delicate.

Laboratory exercise No. 43.—*Testis.* Harden in alcohol, embed in paraffin, and stain with lithium carmine, method No. 3. Demonstrate from your section the investing tunics (vaginalis, albuginea, and vasculosa), lobules, septa, interlobular connective tissue, parietal cells, mother cells, spermatoblasts, and spermatozoa. Drawings.

II. FEMALE REPRODUCTIVE ORGANS.

The female reproductive organs are the ovary, parovarium, Fallopian tubes, uterus, vagina, genitalia, and mammary glands

THE OVARY.

The ovaries correspond to the testes, are oval-shaped, and are situated one on each side of the uterus. The following outline exhibits their structure:

OUTLINE OF THE OVARY.

- Cortex
  - Tunica albuginea.
  - Stroma.
  - Graafian follicles.
    - Theca folliculi.
    - Membrana granulosa.
    - Discus proligerus.
    - Ovum
      - Zona pellucida.
      - Vitelline membrane.
      - Vitellus.
      - Germinal vesicle.
      - Germinal spot.
  - Corpus luteum.
- Medulla
  - Stroma.
  - Blood vessels.

The *tunica albuginea* is a serous coat derived from the peritoneum. The **cortex** includes the outer one-third of the ovary; and the medulla, the inner two-thirds. The *stroma* is the connective tissue ground substance. It contains numerous spindle-cells and occurs in both medulla and cortex. The characteristic structures of the

cortex are the *Graafian follicles,* each of which consists of the theca folliculi, composed of an outer and inner coat; the membrana granulosa, comprising several layers of polyhedral cells; the discus proligerus, the zone of cells surrounding the ovum; and the ovum, comprising the zona pellucida, an investing membrane; vitelline membrane, the cell-wall of the ovum; vitellus, the protoplasm of the ovum, which occupies the space between the vitelline membrane and the nucleus; germinal vesicle, which corresponds to the nucleus; and the germinal spot, or nucleolus.

The number of Graafian follicles in the two ovaries of a child is estimated to be 70,000. The liquid substance within the follicle is the liquor folliculi. When the ovum is ripe it escapes by the bursting of the wall of the follicle, which has gradually approached the surface of the ovary. This makes it possible for the ovum to reach the outside of the ovary, and pass thence to the Fallopian tube. The ovum and Graafian follicle are developed from the germ epithelium upon the surface of the ovary. As they develop, they gradually pass into the deeper portion of the cortex. Upon the escape of the ovum, the ruptured follicle becomes filled with polyhedral cells, which are penetrated by capillaries, and this stage is the *corpus luteum.*

The **medulla** comprises the connective tissue stroma and blood-vessels.

THE PAROVARIUM is of fœtal origin, and is a remnant of the Wolffian body.

THE FALLOPIAN tubes, or oviducts, convey the ova from the ovary to the uterus. Each tube is composed of three coats—(1) the mucous coat, comprising a single layer of ciliated epithelial cells, a tunica propria of connective tissue, muscularis mucosæ, and a small amount of sub-mucous tissue; (2) muscular coat, comprising circular and longitudinal layers; (3) serous coat, derived from the peritoneum, and composed of loose bundles of connective tissue.

THE UTERUS.—This is a continuation of the Fallopian tubes and presents three coats—mucous, muscular, and serous. The mucous coat consists of a fibrous tunica propria and a simple layer of ciliated epithelium. Toward the cervical end, however, the mu-

cosa is thicker, is beset with papillæ, and its surface is lined with stratified squamous epithelium. The muscular coat is dense and is disposed in three layers—inner, middle, and outer—with an intermingling of blood-vessels, nerves, lymphatics, and areolar tissue.

THE VAGINA.—This structure possesses a mucosa, sub-mucosa, and muscular and serous coats. The mucosa is studded with papillæ, and is lined with stratified squamous epithelium; the sub-mucosa consists of fibrous bundles and elastic fibres; the muscular coat is in two layers; the serous coat comprises elastic fibres and endothelium.

The mammary glands are sebaceous racemose glands, consisting of lobes, lobules, and acini, with ducts, connective tissue, and areolar and adipose tissues.

Laboratory exercise No. 44.—*The ovary.* Harden in alcohol, embed in paraffin, and stain with hæmatoxylin and eosin. Demonstrate the following: Cortex, composed of tunica albuginea, stroma, Graafian follicles, and corpus luteum; the medulla, consisting of the stroma and blood-vessels. Make a study of a Graafian follicle, noting the theca folliculi, membrana granulosa, discus proligerus, and ovum. Focus with H. P. upon a ripe ovum and observe the vitelline membrane, vitellus, germinal vesicle, and germinal spot. Drawings.

Laboratory exercise No. 45.—*Fallopian tube* and *uterus.* These may be hardened in alcohol, embedded in paraffin, and stained with lithium carmine, method No. 3. Make out (and illustrate in your diagrams) four characteristic differences between these organs. How are the cilia disposed?

MEMORANDA.

GENITAL ORGANS.

A. The Testis. B. The Ovary.

A. Testis: (a) Tunica albuginea; (b) Mediastinum; (c) Septa; (d) Lobules; (e) Convoluted tubule; (f) Parietal cells; (g) Mother cells; (h) Spermatoblasts; (i) Spermatozoa; (j) Basement membrane; (k) Tubuli recti; (l) Rete testis.

B. Ovary: (a) Tunica albuginea; (b) Medulla, containing stroma and blood vessels: (c) Cortex; (d) Stroma; (e) Graafian follicle; (f) Theca folliculi; (g) Membrana granulosa; (h) Discus proligerus; (i) Ovum; (j) Corpus luteum.

Ovum: (a) Zona pellucida; (b) Vitelline membrane; (c) Vitellus; (d) Germinal vesicle; (e) Germinal spot.

A. Fallopian Tube. B. Uterus.

A. Fallopian Tube: (a) Mucosa; (b) Ciliated epithelium; (c) Tunica propria; (d) Muscularis mucosæ; (e) Submucosa; (f) Circular muscle layer; (g) Longitudinal muscle layer; (h) Serous coat.

B. Uterus: (a) Ciliated or squamous epithelium; (b) Tunica propria; (c) Uterine glands; (d) Folds; (e) Muscular coat; (f) Serous coat; (g) Endothelium.

CHAPTER XXI.

EYE, EAR, AND NOSE.

The organs of special sense are developed from the epiderm. Their nervous supply is received from the cerebro-spinal system. Only a brief discussion of the eye, ear, and nose is here given.

THE EYE.

There are three coats of the eye—an external fibrous tunic, a middle vascular tunic, and an inner nervous tunic.

1. The external coat includes the cornea and sclerotic membrane.

The *cornea* is composed of five layers—(1) The anterior epithelium; (2) the anterior limiting membrane; (3) the substance proper; (4) the posterior limiting membrane; (5) the posterior endothelium.

The *sclerotic* membrane, or sclera, resembles the substantia propria of the cornea and consists of two structures—(1) bundles of white fibrous tissue; (2) a layer of loose connective tissue, the *lamina supra choroidea.*

2. The middle tunic comprises the choroid, ciliary body, and iris.

The *choroid* consists of three layers—(1) the choroidal stroma, containing blood-vessels; (2) the capillary networks; (3) the vitreous membrane.

The *ciliary body* comprises three portions—(1) the ciliary ring; (2) the ciliary processes; (3) the ciliary muscles.

The *iris* comprises the following structures: (1) The anterior endothelium; (2) the anterior boundary layer; (3) the vascular layer; (4) the posterior boundary layer; (5) the pigment layer.

3. The nervous tunic.—This tunic comprises the *retina,* which consists of ten layers—(1) the pigment layer; (2) the layer of rods and cones; (3) the external limiting membrane; (4) the outer nuclear layer; (5) the outer reticular layer; (6) the inner nuclear layer; (7) the inner reticular layer; (8) the ganglion-cell layer; (9) the nerve fibre layer; (10) the internal limiting membrane.

THE EAR.

The specialized neuro-epithelium of the ear is found in the internal ear. It comprises two kinds of cells—the sustentacular cells and the hair cells.

THE NOSE.

The neuro-epithelium of the nose consists of elongated cells with spherical nuclei.

Laboratory exercise No. 46.—*The eye.* Fix in chromic acid, harden in alcohol, and stain with carmine, method No. 3. Make a study of the structures above enumerated, and as far as possible demonstrate the layers of each coat. What is the character of the crystalline lens? The vitreous humor is the same in character as the jelly of Wharton—i. e., mucous tissue.

MEMORANDA.

PART III.

BACTERIOLOGY.

Bacteriology deals with minute vegetable organisms known as bacteria, microbes, germs, etc. The science is comparatively new, and while it is suggested in some quarters that it has accomplished naught of practical value except the discovery of the bacillus of consumption, thereby emphasizing the importance of properly disposing of tubercular sputum, yet this alone is adequate compensation for the labor thus far bestowed. But this is not all. The workers in this field have discovered the specific cause of a number of other diseases, the means of preventing the spread of these diseases, and certain remedies of inestimable value—such as the antitoxin for diphtheria. The results of future investigations will probably exceed the hopes of the most sanguine.

CHAPTER XXII.

CHARACTERISTICS AND CLASSIFICATION OF BACTERIA.

Characteristics.—Bacteria are unicellular, non-nucleated, vegetable organisms, which reproduce by normal fission and by endospores. With but three exceptions, they are devoid of chlorophyl. They are parasitic and saprophytic. Many infest the human body, some producing infectious diseases, while others are supposed to aid in the processes of digestion and assimilation. They assist in the decomposition of dead organic substances, thus performing an inestimable service to man. They reproduce by normal fission and the formation of endospores. Normal fission is accomplished by the division of the protoplasm and the formation of a partition between the two halves. As the cells continue to multiply, they often cohere, thus forming a filament. In other cases the cells are held together in masses, called *zoöglœæ*. An endospore is formed some-

times in the middle of the rod, which becomes swollen centrally, and the spindle-shaped cell is called a *clostridium*. Often the spore occurs in the end of the cell; again the whole cell may assume the function of a spore, giving rise to the so-called *arthrospore*. Spores are designed to perpetuate the species and to this end offer great resistance to external influences, such as cold, heat, antiseptics, etc. Dr. Curtis says: "*Bacillus anthracis* retains its vitality for some time in absolute alcohol and withstands boiling in nutrient solutions for an hour, and has germinated after three years' confinement in dry air."

Classification of bacteria.—The present system of classification is considered imperfect. The outline given below, while not complete, will give a fair idea of the scheme of classification considered most satisfactory by good authorities:

Classification:

Kingdom—Vegetable.
Series—Cryptogamia.
Sub-kingdom—Thallophyta.
Class—Fungi.
Sub-class—Schizomycetes.

Sub-class.	Group.	Family.	Genus.
Schizomycetes, or Bacteria	Monomorphous	Coccaceæ (spherical)	Micrococcus.
			Streptococcus.
			Diplococcus.
			Tetracoccus.
			Staphylococcus.
			Ascococcus.
			Sarcina.
		Bacteriaceæ (rod-shaped)	Spirillum.
			Vibrio.
			Spirochœte.
			Bacillus.
			Proteus.
			Bacterium.
	Pleomorphous	Spirulineæ	Spirulina.
		Leptotricheæ	Leptothrix.
			Beggiota.
			Crenothrix.
			Phragmidiothrix.
		Cladotricheæ	Cladothrix.

CHAPTER XXIII.

MORPHOLOGY, KINDS, AND PRODUCTS.

MORPHOLOGY.

The present classification of bacteria depends largely upon their forms. When a species assumes but one form, it is said to be *monomorphous;* but if it possesses several forms, it is *pleomorphous.*

Monomorphous bacteria are represented by three important divisions: **Cocci,** spherical cells; **Bacilli,** or rod-shaped bacteria; and **Spirilla,** or curved bacteria. Cocci are represented by several genera—*Micrococcus,* in which the cells occur singly; *Diplococcus,* in which the cells occur in pairs, the result of binary division; *Tetracoccus,* in which the cells occur as tetrads; *Sarcina,* in which the cells occur in cubes, or packets; *Streptococcus,* where the cells occur in chains, or filaments, the result of fission in one direction; *Staphylococcus,* in which the cells occur in masses, like a cluster of grapes; *Ascococcus,* in which the cocci are in globular masses.

Spirilla include the genera Spirillum, Vibrio, and Spirochœte. The elements are curved, sometimes comma-shaped, and again in long spirals.

Bacilli are represented by the genera *Bacillus, Proteus,* and *Bacterium.* The species of these genera are illustrated by rod-shaped cells. The rods may be pointed at the ends, as in the *clostridium;* truncate, as with *anthracis;* round, as with *subtilis.* Some form filaments, as *anthracis;* others are always single, as *pyocyanus.* Some are slender, as *tuberculosis;* others, large and thick, as *subtilis.* Involution forms occur where the rod deviates from the characteristic form, due to external conditions or the death of the cell.

Pleomorphus bacteria are represented by *Spirulina, Leptothrix,* and *Cladothrix.* Among these the individuals of each species assume more than one form.

KINDS OF BACTERIA.

The following are the important kinds of bacteria:

1. **Parasitic,** those depending for subsistence upon a living host.

2. **Saprophytic,** those subsisting upon dead organic matter.

3. **Aërobic,** those requiring oxygen.

4. **Anaërobic,** those subsisting without oxygen.

5. **Facultative,** those that are anaërobic, but may become aërobic; and those saprophytic that may become parasitic.

6. **Pathogenic,** those producing diseases in man and animals.

7. **Chromogenic,** those producing pigments.

8. **Zymogenic,** those producing fermentations.

9. **Motile,** those having independent motion.

10. **Liquefying,** those that produce a ferment which liquefies solid nutrient gelatin.

PRODUCTS.

Bacteria are powerful agents in breaking up complex chemical substances into simple ones. They attack the tissues of living organisms and produce the toxins and ptomaines which cause the symptoms of destructive diseases. They assist animals in destroying dead organic matter, else the earth would be covered with its dead. Thus, by their coöperation with other humble organisms, they make life upon the earth possible. They assist in manufacturing articles of economic value, such as butter, cheese, etc. They convert starch into sugar, dissolve cellulose, change albumin from an insoluble to a soluble form, convert urea into ammonium carbonate, produce lactic acid in milk, produce acetic acid from alcohol, and manufacture bright pigments.

The pigment formed by *Bacillis prodigiosus* was once supposed by the superstitious to be blood formed by supernatural power. Certain gaseous products are sometimes evolved by the action of bacteria. The foul odors from putrefying bodies may be ascribed to their action. Among the common gases which are liberated from organic substances by the agency of bacteria may be named nitrogen, carbon di-oxide, and sulphuretted hydrogen. The demonstration of gaseous products may be made by the growth of certain species in nutrient media in a saccharometer. Indol is produced by some species. The test for this substance is made by adding to the peptone solution in which the bacteria have been vegetating for twenty-four hours about ten drops of c. p. sulphuric acid. The development of a

rose-color indicates the presence of indol and nitrites. Should no rose-color appear, another tube should be tested, adding, first, 1 c. c. of .01 per cent solution of sodium nitrite, and then the sulphuric acid. The formation of a rose-color indicates the presence of indol alone.

Laboratory exercise No. 47.—*Morphology.* Make cover-glass preparations of species of cocci, bacilli, and spirilla. Note the peculiar form of the individuals of each species. To demonstrate the different kinds of cocci, the following species may be used: Micrococcus ureæ, Diplococcus pneumoniæ, Sarcina lutea, Streptococcus pyogenes, Staphylococcus pyogenes aureus, and Ascococcus Billrothii.

Apply to the surface of a cover-glass some of the scrapings obtained from the teeth just under the gums. Cover this with another glass and by pressure spread out the material so as to make a thin film. Dry, fix, and stain. (See method No. 10.) Examine your preparation and observe cocci, spirilla, and bacilli. Note the large ribbon-like forms, which probably represent *Leptothrix buccalis.* Make drawings to illustrate all of these forms.

Laboratory exercise No. 48.—*Motility.* Apply to a clean slide some of the scum upon the surface of hay infusion. Cover and note the slender filaments made up of rod-like cells. Each cell represents an individual of *Bacillus subtilis.* Observe the slow, gliding motion of the filaments. How is this motion secured? Look for other bacteria. Some may be found with a vibratory motion. If this motion is confined to one place without producing any progress across the field of vision, it is purely physical, the so-called Brownian movement.

Laboratory exercise No. 49.—*Products.* Fill the long arm of the Saccharometer with bouillon and inoculate the medium with some zymogenic species. The formation of gases in the upper end of the arm indicates the action of the bacteria. Make the test for indol, as described above, using as a culture-medium Dunham's Peptone solution, which is prepared by boiling, filtering, and sterilizing a mixture of 10 grams of peptone, 5 grams of sodium chloride, and 1 liter of water.

FORMS OF BACTERIA.

A. Cocci. **B. Bacilli.**

A. Cocci: (a) **Micrococcus**; (b) **Diplococcus**; (c) **Tetracoccus**; (d) **Sarcina**; (e) **Streptococcus**; (f) **Staphylococcus**; (g) **Ascococcus**; (h) **Zoöglœa.**

B. Bacilli: (a) **Single cells**; (b) **Filaments**; (c) **Cells with round, pointed, and truncate extremities**; (d) **Polar spore**; (e) **Clostridium with central spore.**

C. Spirilla. **D. Pleomorphous Forms.**

C. Spirilla: (a) **Spiral filament**; (b) **Comma-shaped cells.**

D. Pleomorphous forms: (a) **Spirulina**; (b) **Leptothrix**; (c) **Cladothrix.**

CHAPTER XXIV.

SIZE, NUMBERS, AND DISTRIBUTION.

Size and numbers.—The unit of measurement for bacteria is the micromillimeter, which is the thousandth part of a millimeter. It is represented by the Greek letter μ. The size of *Bacillus tuberculosis* is: Length, 1.5 μ to 4 μ; diameter, 0.2 μ to 0.5 μ. Some cocci are as small as 0.15 μ in diameter; others as large as 2.8 μ in diameter. Bacilli range from 1 x 0.2 μ to 5 x 1.5 μ. Some spirilla are 40 μ long. The number of known species is upward of 1,000 The individuals of any species are countless. Dr. Sternberg states that a milligram of the pure culture of *Staphylococcus pyogenes aureus* contains 8,000,000,000 individuals. The weight of an average bacterium has been estimated by Nageli to be 1-10,000,000,000 milligram.

The following estimates, as given by Williams, will give some conception as to the number of bacteria that form a part of the invisible world of teeming organisms. The number of individuals in 1 c. c. of virgin soil is estimated to be upward of 100,000. Ordinary milk contains more than 20,000 to 1 c. c. The number of bacteria in a milligram of human fecal matter will range from 70,000 to 33,000,000.

Distribution.—The estimates just given will suggest that bacteria are very widely distributed. Their spores float upon the dust of the atmosphere in untold millions; they swarm in the water we drink; they teem in the soil to the depth of three feet; they abound in food and in all decaying substances; and they take up their abode in the human body, being present in the alimentary tract in large numbers, except in new-born infants. It is believed that the air upon the high seas and upon mountain tops, the deeper layers of the soil, the water of uncontaminated springs, and the tissues and fluids of the normal body which are not exposed to the external atmosphere are free from their presence.

The presence of bacteria in soil, air, water, milk, blood, urine, and fecal matter may be demonstrated by making plate cultures

from the substances to be tested. If properly prepared the plate will exhibit as many colonies, approximately, as there were individuals in the known amount of the material used. This furnishes a working basis for counting the number of specimens in a given quantity of any substance. By exposing a sterilized Petri dish containing agar media to the air for a few moments and then covering, in a day or so many colonies will appear, indicating the kinds and number of species which occur in the atmosphere. By plating, these can be isolated, studied, and classified.

While in normal condition the human body is comparatively free from bacteria, yet the alimentary and respiratory tracts always contain large numbers, and, under abnormal conditions, the tissues, blood, lymph, and urine often become infested. The products of the mucous membrane (especially the hydrochloric acid) and the serum of the blood, which are germicidal, as well as the phagocytes, are generally effectual checks to the invading hosts.

The outer layers of the skin furnish the abode of many species, and it is claimed to be impossible to dislodge all the germs that occur under the nails.

There are certain agencies which influence the growth and distribution of bacteria. Electricity arrests growth. Sunlight will kill *Bacillus tuberculosis* and other species, a few hours of sunlight being sufficient to kill the vegetative cells of *Bacillus anthracis*. Acids and oils are usually antiseptic. Some species require oxygen, while others will not grow in its presence. Many species can withstand severe cold, but quickly succumb to a high temperature. The temperature at which a species dies is called its thermal death point. The thermal death point of a considerable number of species is about 60 degrees Centigrade. There is a certain temperature most favorable to the growth of each species. This for many species is about 35 degrees Centigrade.

Laboratory exercise No. 50.—*Bacteria in air, water, soil, etc.* Expose to the air the agar-agar in a sterilized Petri dish for a few moments. Examine in a day or so and observe and count the colonies upon the surface of the medium. Note that they differ in form and color. Inoculate five test-tubes of agar-agar, one with each of the following substances: Soil, ordinary drinking water, milk, urine, and saliva. These materials should be collected in sterilized containers. Label each tube, and in a few days examine for any growths which may have appeared.

CHAPTER XXV.

CULTIVATION AND SYSTEMATIC STUDY OF BACTERIA.

CULTIVATION OF BACTERIA.

This is accomplished by the use of certain media upon which the species to be cultivated will grow. Such are potato, blood serum, gelatin, and agar-agar. The medium, when prepared, is placed in cotton-stoppered tubes and then sterilized. In the case of gelatin and agar-agar, a steam sterilizer can be used, and sterilization should be made on three successive days, from fifteen minutes to one hour each day.

CULTURE MEDIA.

The following formulas for the preparation of culture media will be useful:

1. Meat broth.—To one liter of water add one pound of finely-chopped lean meat, free from all fat. Let stand over night, or heat for one hour, but do not boil. Filter.

2. Bouillon. —To one liter of meat broth add ten grams of peptone and five grams of sodium chloride. The peptone and sodium chloride should be thoroughly mixed in a mortar with water, until a thin paste is formed, before adding them to the meat broth. Cook one hour, filter, and alkalize with a solution of sodium carbonate. Sterilize.

3. Agar-agar. —To one liter of bouillon add fifteen grams of agar. Cook in sterilizer, or double sauce-pan, until agar is dissolved, one to three hours. Neutralize with solution of c. p. $Na_2 Co_3$. Filter. Cool to body temperature and add the whites of two eggs, which have been previously mixed with 100 c. c. of water. Cook one hour. Filter with coarse filter to remove coagulated albumen. Heat again and filter with best filter paper previously moistened with boiling water. Should any of the medium fail to filter through the first paper, it should be heated again, and a second paper used for that portion. Fill tubes and sterilize on three successive days. Cool with tubes in oblique position.

4. Nutrient gelatin.—To one liter of bouillon add 100 grams of finest gelatin. Heat twenty minutes in steam sterilizer. Alkalize with solution of c. p. sodium carbonate and filter with filter paper in steam sterilizer. Fill tubes. Sterilize for three successive days, fifteen minutes each day.

5. Blood serum.—Collect blood in a sterilized container. When coagulated, loosen clot and let stand twenty-four hours in a cool place. Remove clear serum and fill sterile test-tubes with the same. Coagulate at 65 degrees to 75 degrees Centigrade. Sterilize at 58 degrees Centigrade, one hour each day for six days.

6. Potato medium.—After thoroughly washing potatoes in soap and water and removing eyes and spots, immerse in mercuric chloride solution, 1 to 1,000, for ten minutes. Make cylinders of the potatoes the size of tubes; bisect obliquely, placing each half in a tube. Sterilize for three successive days, a half hour each day.

As already suggested, each medium, when prepared, is to be placed in cotton-plugged tubes, and then sterilized. The cotton proves a perfect filter for the bacteria. Agar-agar may be kept for months without any indication of growth of any kind, the cotton plug preventing all access of germs from the outside.

INOCULATION.

The inoculation of the media is accomplished by means of a sterilized platinum wire. A small portion of the pure culture of the species desired is caught upon the loop at the end of the wire and then drawn over the surface of the medium. Care should be taken to sterilize the wire both before and after using.

PLATING.

A pure culture of a species is obtained by plating. This is accomplished by gently heating, until melted, the nutrient gelatin, or agar-agar, in three tubes. With a sterilized platinum wire, a small portion of the substance containing the bacteria is transferred to the liquefied gelatin, or agar-agar, in tube number *one*. Then, after sterilizing the wire again, a small quantity of the contents of tube *one* is transferred to tube *two*. In like manner, tube *three* is inoculated from tube *two*. Then the contents of the tubes are trans-

ferred to three sterilized Petri dishes. After numbering and labeling, the dishes are set aside for future examination. If colonies occur on plates *two* and *three,* they are probably pure cultures of the species desired. From these colonies tubes containing media can be inoculated.

HANGING-DROP CULTURES.

It is often very desirable to make a microscopic study of bacteria and other organisms in the process of growth. This is accomplished by resorting to the hanging drop culture. A small drop of the liquid media containing the species to be studied is transferred by a platinum loop to the center of a sterilized cover-glass. This is then inverted upon a slide in the center of which a concavity has been ground out. The edges of the cover-glass are then sealed with a layer of vaseline applied with a camel's-hair brush. The preparation may then be studied from time to time, the focusing being applied to the edges of the preparation, rather than the center, which will generally be found opaque.

INOCULATION OF ANIMALS.

To save human life it is often quite necessary to sacrifice the lives of lower animals. The experiments of bacteriologists, while appearing to the superficial observer as almost merciless, are in the interests of the highest humanity and are destined not only to diminish in a large degree the sum of human suffering, but to bring alleviation to lower animals, at whose expense the requisite knowledge is sought. The life of man outweighs that of a mouse or "many sparrows." For purposes of experimentation, such animals as mice, rats, guinea pigs, and rabbits are generally employed. Inoculations are made in the ear, at the root of the tail, and elsewhere. The hair is first removed by cutting or searing. A V-shaped incision is then made, and the infected material inserted by means of a platinum wire. Inoculation of the blood may be made with a bacteriological syringe.

SYSTEMATIC STUDY OF BACTERIA.

The identification of any species can only be made after a thorough study of its characteristics. Even then the determination will

sometimes be attended with considerable difficulty and doubt. In the systematic study of an unknown species, the following outline may prove of service: (1) Name, (2) habitat, (3) growth on media, (4) temperature, including that of most favorable growth and the thermal death point, (5) relation of growth to oxygen, (6) gas formation, (7) chemical reaction, (8) formation of indol, (9) pigmentation, (10) pathogenesis, (11) aniline reaction, (12) motility, (13) morphology, (14) size, (15) spore formation.

When these tests have been made, the classification may be determined by using analytic keys, such as are found in the valuable works of Sternberg and Crookshank.

Laboratory exercise No. 51.—*Culture Media.* Let the student prepare or assist the instructor in preparing the following media: Bouillon, agar-agar, blood serum, nutrient gelatin, and potato medium. Make inoculations upon agar-agar as directed above. The tubes should be held in the left hand, between the thumb and forefinger, in such a way that the palm of the hand will be vertical and the tubes but slightly inclined. The cotton plugs, when removed, may be held between the fingers. The greatest care should be exercised, always sterilizing the platinum needle before and after using. Make a stab-culture of some anaërobic species. This is accomplished by holding the tube in a vertical position, using a platinum wire with small loop, and plunging this through the center of the medium from the surface to the bottom of the tube.

Experiments with animals. Clip the hair from the base of the tail of a mouse. Make a V-shaped incision and insert into the wound some of the saliva of the mouth. Saliva often contains *Diplococcus pneumoniae*, which will cause the death of an inoculated mouse in a few hours. Other inoculations may be made as desired.

Laboratory exercise No. 52.—*Hanging-drop Cultures.* Prepare a hanging-drop culture upon which have been sown some spores of any species of mold, such as *Penicillium glaucum*. After a few days examine. The spores of molds may sometimes be mistaken for cocci. The hyphæ, or slender filaments, which compose their structure, may, when broken into fragments, have some resemblance to the cells of bacilli. The hyphæ develop from the spores, producing three characteristic portions—the root hyphæ; the mycelium, or prostrate portion; and the ærial hyphæ, upon the extremities of which the sporangia containing the spores are developed. Also make hanging drop cultures of species of bacteria, and study the same from time to time.

Laboratory exercise No. 53.—*A systematic study of Bacteria.* Make a systematic study of *Bacillus prodigiosus* and other common species according to the method suggested above. Fill out the outline on page 155 and make drawings of agar cultures and the microscopic elements.

OUTLINE FOR SYSTEMATIC STUDY.

Species .....

1. Habitat .....
2. Growth on media—
   (1) Gelatin .....
   (2) Agar-agar .....
   (3) Blood-serum .....
   (4) Potato .....
3. Relation to temperature—
   (1) Best growth .....
   (2) Thermal death-point .....
4. Relation to oxygen .....
5. Gas formation .....
6. Chemical reaction .....
7. Formation of indol .....
8. Pigmentation .....
9. Pathogenesis .....
10. Anilin reaction (Gram's method) .....
11. Motility .....
12. Morphology .....
13. Size .....
14. Spore formation .....
15. Classification .....

CHAPTER XXVI.

MICROSCOPIC TECHNIQUE.

I. REAGENTS AND STAINS.

(1) DECOLORIZING SOLUTIONS.

Twenty-five per cent aqueous solutions of hydrochloric, nitric, and sulphuric acids may be used for decolorizing.

(2) ACID ALCOHOL.

Hydrochloric acid..... 1 part
Alcohol (seventy per cent)..... .....100 parts

(3) IODINE SOLUTION.

Iodine..... 1 gram
Potassium iodide..... 2 grams
Water..... .....300 cc.

(4) CARBOL FUCHSIN.

Fuchsin..... 1 cc.
Alcohol..... .....10 cc.

Dissolve and add 10 c. c. of five per cent solution of carbolic acid. Filter.

(5) ACID METHYLENE BLUE.

Sulphuric acid..... ...16 cc.
Water..... .....90 cc.
Methylene blue..... 2 grams

This stain should be prepared fresh from time to time. The carbol fuchsin improves with age.

(6) LÖFFLER'S ALKALINE METHYLENE BLUE.

Concentrated alcoholic solution of methylene blue..... 30 c.
Potassum hydrate (Aq. Sol., 1-10,000).....100 cc.

This is especially useful in staining the baccillus of diphtheria.

(7) ANILINE-WATER GENTIAN-VIOLET.

Aniline oil ..... 5 cc.
Water..... .....100 cc.

Mix, shake vigorously, filter; the fluid after filtration should be perfectly clear; add

Alcohol	10 cc.
Alcoholic solution of gentian-violet	11 cc.

This solution should be freshly prepared about every two weeks.

(8) LÖFFLER'S MORDANT FOR FLAGELLA.

Tannic acid	2 grams
Water	8 cc.
Saturated solution of ferrous sulphate	5 cc.
Saturated alcoholic solution of fuchsin	1 cc.

(9) ANILINE-WATER DYE FOR STAINING SPORES.

Saturated alcoholic solution of fuchsin or gentian-violet	11 parts
Aniline oil water	100 parts
Abs. alcohol	10 parts

Keeps well for ten days.

(10) AQUEOUS STAINS.

Saturated aqueous solutions of fuchsin, gentian-violet, and methyline blue will be found useful for all simple staining.

(11) ALCOHOLIC SOLUTIONS.

Saturated alcoholic solutions of fuchsin, gentian-violet, and methyline blue should be kept on hand to be used in simple staining and in connection with other stains.

II. STAINING METHODS.

(1) SIMPLE STAINING.

This consists in using a single stain. The process is given on page 27, method No. 10.

(2) DOUBLE STAINING.

This consists in using two stains, one to stain spores, protoplasm, etc., and the other as a ground stain. The following methods will illustrate double staining:

Method No. XII.—Staining of Spores.

(a) Make a cover-glass spread, dry and pass three times through the flame.

(b) Add aniline-water gentian-violet.

(c) Heat until the preparation begins to boil; remove for a minute. Repeat this process six times.

(d) Wash in three per cent hydrochloric acid-alcohol one minute

(e) Wash in water.

(f) Counter-stain with aqueous methylene blue half a minute.

(g) Wash in water.

(h) Dry and clear up with xylol.

(i) Mount in balsam.

Method No. XIII.—Staining of Flagella.

(a) Mix upon the cover-glass a portion of the culture with a drop of water, using care not to break off the delicate flagella.

(b) Dry and pass three times through a flame.

(c) Apply Löffler's mordant one minute, warming gently.

(d) Wash in water.

(e) Stain with aniline-water fuchsin.

(f) Wash in water.

(g) Dry and mount in balsam.

Method No. XIV.—Gram's Method for Bacteria.

(a) Make a cover-glass preparation by the usual method.

(b) Stain with aniline-water gentian-violet solution, two to five minutes, warming slightly.

(c) Add Gram's iodine solution one and one-half minutes.

(d) Apply alcohol, repeatedly, as long as stain continues to come away from the preparation.

(e) Wash in water and examine as a water-mount.

(f) If desired, dry and mount in balsam.

Method No. XV.—Gabbet's Method for Tuberculosis, etc.

(a) Make a cover-glass smear of the sputum, pus, blood, or urine to be examined. After the preparation is dry, affix by passing three times through the flame.

(b) Using a Cornet forceps, apply carbol-fuchsin five to ten minutes, heating until steam appears.

(c) Wash in water.

(d) Apply alkaline methylene blue for one minute.

(e) Wash in water.

(f) Dry and mount in balsam.

Staining Tissues for Bacteria.

Tissues may be stained by Gram's method or by the following process:

Method No. XVI.—Method for Staining Bacteria in Sections.

(a) Using an aqueous solution of fuchsin, gentian-violet or methylene blue, apply stain for about five minutes.

(b) Wash in water.

(c) Apply an aqueous solution of acetic acid, one per cent, for one minute.

(d) Apply alcohol one to two minutes.

(e) Clear up with xylol.

(f) Mount with balsam.

MEMORANDA.

CHAPTER XXVII.

NON-PATHOGENIC BACTERIA.

Material for the practical study of non-pathogenic species may be obtained from air, water, soil, and other sources. The biological characteristics of a few of the more common species are here given to assist the student in experimental work.

I. Bacillus Prodigiosus.

This species is a chromogenic, non-motile, facultative anaërobic saprophyte. It produces a pigment-forming body, which becomes red by the action of oxygen. The pigment gives rise to the "red mould" of bread. The rods are short, often in filaments, without spores. It grows rapidly upon agar-agar, or potato, at the room temperature, and soon liquefies nutrient gelatin. It grows best at 25 degrees Centigrade. It may be obtained from the air.

II. Bacillus Acidi Lactici.

Bacillus acidi lactici occurs in sour milk, producing lactic acid. It is a non-motile, facultative anaërobic saprophyte. It produces a whitish growth on agar-agar, does not liquefy gelatin, and the rods occur in pairs or short filaments, producing large shining polar spores. It causes milk to sour, changing its sugar into lactic acid and CO_2, and precipitates casein. It will grow at 10 degrees Centigrade; but, when cultivated for several generations, it loses its power to produce fermentation.

III. Bacillis Subtilis.

This species may be obtained from hay infusions, air, water, soil, etc. It produces a grayish growth on agar, and liquefies gelatin. It is a motile aërobic saprophyte, and grows rapidly at ordinary temperatures. The rods are thick and stout, with rounded extremities, and provided with flagella. It produces motile spores.

IV. Bacillus Violaceus.

This bacillus is found in water. It is aërobic, motile, and chromogenic; grows at room temperature, and on agar produces a violet-

colored covering which lasts for years. The rods are slender, with rounded ends, and produce small oval spores. It grows upon agar and liquefies gelatin.

V. Proteus Vulgaris.

Proteus vulgaris is found in putrefying animal matter; is a facultative anaërobic motile saprophyte; has rods with rounded ends, which grow into flexible filaments; produces a whitish growth on agar, and liquefies gelatin; forms H_2S, and causes putrefaction, occasionally being pathogenic to man.

VI. Micrococcus Ureæ.

This species may be obtained from cystitic and decomposing urine. The cocci occur singly, in pairs, or in filaments. It is an aërobic saprophyte, grows readily at room temperature, and does not liquefy gelatin. Plate cultures appear like a drop of wax upon the surface.

VII. Sarcina Lutea.

Sarcina lutea may be obtained from the air. It is an aërobic chromogenic species, whose cocci occur in pairs, tetrads, and packets. The pigment is yellow. It liquefies gelatin slowly.

VIII. Spirillum Rubrum.

This is a motile chromogenic facultative anaërobic species. It may be found in the putrefying cadaver of a mouse. The spirals make three-quarter turns. It grows on agar, stab cultures, forming a red pigment.

Laboratory exercise No. 54.—*Bacillus subtilis.* Prepare a pure culture of this species and inoculate tubes of agar and gelatin. Make a study of the growths upon these media, describing each. Make a water-mount and demonstrate the motility of the rods and filaments. Make a cover-glass preparation and stain with gentian-violet, method No. 10. Demonstrate flagella, staining by method No. 13. Demonstrate spores by method No. 12. Prepare an outline of this species as indicated on page 155. Make drawings of cultures and rods.

Laboratory exercise No. 55.—*Micrococcus ureae.* Obtain plate cultures from decomposing urine. Note the wax-like colonies. Make a cover-glass preparation and stain with gentian-violet. Observe the spherical cells arranged singly, in pairs, and in chains. How does this species

affect urea? Prepare an outline, as with the last species, and make drawings of cultures and cells.

Laboratory exercise No. 56.—*Sarcina lutea.* Expose agar-agar in a Petri dish to the air, and from the yellowish colonies which develop prepare plates. Inoculate tubes (from the pure cultures thus obtained) of agar and gelatin. Describe the growth in each tube. Stain by method No. 10. Note the cocci, arranged singly, in pairs, in tetrads, and in packets. Make drawings of cultures and cells.

Laboratory exercise No. 57.—*Spirillum rubrum.* Obtain a pure culture of this species. Prepare a cover-glass spread, and stain with gentian-violet, method No. 10. Note the spirals and count the turns in each. Drawings.

Note.—Other species may be substituted for those given above, and additional ones may be required.

MEMORANDA.

NON-PATHOGENIC BACTERIA.

A. Bacillus Subtilis.

B. Micrococcus Ureæ.

A. Bacillus subtilis: (a) Culture tube; (b) Cells.
B. Micrococcus ureæ; (a) Culture tube; (b) cells.

A. Sarcina Lutea.

B. Spirillum Rubrum.

A. Sarcina lutea: (a) Culture tube; (b) Cells.
B. Spirillum rubrum: (a) Culture tube; (b) Cells.

CHAPTER XXVIII.

PATHOGENIC BACTERIA.

In all practical work with pathogenic bacteria, more than ordinary care should be used. While the danger attending such work should not be unduly magnified, the student will do well to attend carefully to any abrasions on the skin; he should be scrupulously neat and cleanly, not allowing the material used to be carelessly scattered; he should dispose of all material as soon as used, and carefully cleanse the hands at the close of each period. It is doubtless true that *Bacillus mallei* has caused the death of more bacteriologists from accidental infection than all other species together. The diseases known to be produced in man by bacteria are tuberculosis, leprosy, glanders, anthrax, tetanus, erysipelas, gonorrhœa, pneumonia, influenza, diphtheria, typhoid fever, Asiatic cholera, relapsing fever, malignant edema, bubonic plague, and suppuration. It is believed that some kind of micro-parasite will be found to be the specific cause of each of the following diseases—viz., syphilis, mumps, smallpox, chicken pox, measles, scarlet fever, yellow fever, whooping cough, and others. The limited space of this Manual will allow but a brief discussion of a few of the more important species.

I. Bacillus Tuberculosis.

This species is the recognized cause of consumption, or tuberculosis. It is a non-motile facultative saprophyte, and consists of slender, beaded staves. It may be cultivated on glycerine agar-agar, growing best at 37 degrees Centigrade. It reproduces by fission, and probably by spore-formation. It is also pathogenic to a number of animals. Man may become infected through wounds, through nutrition—such as the milk of tuberculous cows—and by inhalation. The sputum of the consumptive, if not properly destroyed, dries and becomes pulverized. As dust, it floats in the atmosphere, is inhaled, and under suitable conditions produces infection. It is doubtful whether any one is immune from the disease.

Tuberculin is prepared by concentrating with heat the glycerine-bouillon, containing an old growth of *tuberculosis*, and filtering through unglazed porcelain. It is used for the detection of tuberculosis in animals. The suspected animal is injected with the tuberculin, and a sudden rise of temperature, or suppuration of tubercular formations, may be considered as proof that the animal is infected with the disease.

II. Bacillus Typhosus.

The bacillus of typhoid fever is a motile parasite found in the urine and fecal discharges of typhoid patients. The rods have rounded ends, and sometimes grow out into long filaments. It produces a whitish growth on agar, growing best at 35 degrees Centigrade. Spore-formation has not been observed. It may be stained with aqueous solutions of fuchsin, methylene blue and gentian-violet.

The detection of typhoid in a patient may be made by adding to some serum obtained from his blood a quantity of pure culture. If the patient has the disease, the motile germs will soon cease their movements. Flagella may be demonstrated by method No. 13.

III. Bacillus Pyocyanus.

This species is an actively-motile aërobic parasite, presenting very short, slender staves. It is found in green pus, and produces a greenish-white growth on agar-agar at the room temperature. It may be stained with aqueous fuchsin.

IV. Bacillus Anthracis.

This bacillus is the specific cause of anthrax in cattle and the so-called "wool-sorter's disease" in men. It occurs in rods, which have truncate ends (slightly indented), and generally grow out into long filaments. It produces a dry, easily-detached growth on agar, and readily liquefies gelatin. Its movements are rotary. The spores are ovoid, may be central or polar, and are very resisting, having been known to live twenty years. It stains well with aqueous fuchsin or gentian-violet.

V. Bacillus Diphtheriæ.

This is found in diphtheretic membrane. It is a non-motile, aërobic species. On agar it produces a yellowish-white growth, with crenated edges. The rods are straight, or curved, and, when stained, often present the appearance of a dumb-bell, owing to the deeper staining of the polar protoplasm. It grows best at 35 degrees Centigrade, and stains well with Löffler's methylene blue.

The antitoxin of diphtheria is produced by inoculating a horse with a small amount of diphtheria toxin and following this up with an increased dose every six days, until upwards of 1,000 c.c. can be introduced at a time. As a result of this, the serum of the blood becomes immune to the influence of the toxin. A portion of the blood is then removed from the jugular vein of the horse, and, after coagulation, the serum is tested, bottled, and sold in so-called units of strength. A unit of antitoxin has been tersely defined by MacFarland as "ten times the least amount of antitoxic serum that will protect a standard (300-gram) guinea pig against ten times the least certainly fatal dose of diphtheria toxin." A child of the writer was supposed to have diphtheria, bacteriological tests made immediately proving the suspicion to be well founded. Within twenty-four hours of the first appearance of the diphtheretic membrane, 100 units of antitoxin were administered, and in three days the child was considered well.

VI. Staphylococcus Pyogenes Aureus.

This species occurs in suppurations. The cells are spherical in form and occur singly, or in clusters resembling bunches of grapes, called zoöglœæ. It is a non-motile anaërobic facultative parasite. It produces a yellowish growth on agar, grows at the room temperature, liquefies gelatin, and stains well with aqueous solutions.

VII. Streptococcus Pyogenes.

Streptococcus pyogenes is found in erysipeloid suppurations. The cells are spherical, and occur in pairs and chains. It is a non-motile facultative anaërobe, growing best at 35 degrees Centigrade, producing a grayish-white line on agar-agar. It stains well with aqueous fuchsin, or gentian-violet.

VIII. Diplococcus Pneumoniæ.

This is found in normal saliva and in the sputum of croupous pneumonia. The cells are lance-shaped, occurring in pairs, surrounded by a capsule. It is a non-motile facultative saprophyte, and produces round, grayish-white colonies on nutrient gelatin, and is non-liquefying. It may be stained by Gram's method or with the aqueous solutions of aniline dyes. Injected into a mouse, it produces speticæmia.

IX. Bacillus Coli Commune.

The colon bacillus is found associated with *typhosus* in typhoid fever, and with *Micrococcus urcæ* in cystitis. It is recognized as the cause of most of the summer complaints among children, and is almost invariably found in the feces of healthy persons. It is motile, grows luxuriantly on ordinary media; produces acids, gases, and indol; coagulates milk, and does not react with typhoid blood. It may be stained with fuchsin or gentian-violet.

X. Micrococcus Gonorrhœæ.

This microbe is the cause of gonorrhœa. It occurs in gonorrhœal discharges from the urethra, the somewhat hemispherical cells occurring on the surfaces of epithelial cells, or in pus-cells in pairs or tetrads. It is a non-motile facultative anaërobe. The cocci do not stain by Gram's method, but may be stained with Loffler's methylene blue or aqueous solutions of fuchsin and gentian-violet. It does not grow upon gelatin.

Other species which may be studied are the *Bacillus tetani* of tetanus, *Bacillus influenzæ* of influenza, *Baccillus leprœ* of leprosy, *Bacillus mallei* of glanders, *Spirillum choleræ* of cholera.

Laboratory exercise No. 58.—*Staphylococcus pyogenes aureus.* Make a systematic study of this species and write out a full outline according to the form given on page 155. Stain by method No. 10. and study with one-twelfth oil-immersion objective. Make out single cells and a zoöglœa.

Laboratory exercise No. 59.—*Streptococcus pyogenes.* Make a systematic study of this species, preparing an outline and making the required drawings. Stain with aqueous or carbol fuchsin. Observe single cells and slender bead-like filaments.

Laboratory exercise No. 60.—*Micrococcus gonorrhœæ.* Make a cover-glass preparation from gonorrhœal discharges and stain with Löffler's methylene blue or carbol fuchsin, method No. 10. The hemispherical cocci will be found in pairs or tetrads on epithelial cells or within pus cells.

Laboratory exercise No. 61.—*Bacillus tuberculosis.* Make a rather thick smear of tubercular sputum upon a cover-glass, dry thoroughly, and stain by method No. 15. Observe the slender, beaded, somewhat curved rods. Find two attached by their extremities forming a V-shape. Account for the beaded appearance.

Laboratory exercise No. 62.—*Bacillus typhosus.* Make a systematic study of this species and prepare an outline of your work. Observe the motility of vegetative specimens. Demonstrate flagella, method No. 13. Make a permanent preparation, staining with gentian-violet. Look for small oval spaces in the ends of some of the degenerated bacilli.

Laboratory exercise No. 63.—*Bacillus anthracis.* Make a systematic study of this species and prepare an outline. Stain a permanent preparation with gentian-violet. Make a study of the long filaments. Demonstrate spores by method No. 12. Harden, embed, and section the heart and lungs of a mouse that has been killed by *Bacillus anthracis*, and stain by method No. 16. Search for bacteria.

Laboratory exercise No. 64.—*Bacillus coli commune.* Make a systematic study of the colon bacillus. State all the points of difference between this species and *Bacillus typhosus.* Stain your permanent preparation with fuchsin or gentian-violet. Make drawings of all species studied.

Laboratory exercise No. 65.—*Bacillus diphtheriae.* Make a study of cultures of the diphtheria bacillus on different media. Describe the process of making a bacteriological diagnosis of diphtheria. Stain a cover-glass preparation with Loffler's methylene blue and make a study of the cells. Observe the dumb-bell forms. A good lens will always exhibit complete rods, showing that the protoplasm of the polar ends is connected. Search for involution forms, also for three or four cells joined by their extremities, noting that no chains are formed. Drawing.

PATHOGENIC BACTERIA.

Diagrams Showing Cells, Spores, etc.

A. Staphylococcus pyogenes aureus; B. Streptococcus pyogenes; C. Micrococcus gonorrhœæ; D. Bacillus tuberculosis.

Diagrams Showing Cells, Spores, etc.

A. Bacillus typhosus; B. Bacillus anthracis; C. Bacillus coli commune; D. Bacillus diphtheriæ.

CHAPTER XXIX.

IMMUNITY, TOXINS, ETC.—GERMICIDES, ANTISEPTICS, ETC.

Ptomaines.—This term applies to all compounds of a basic nature produced by the agency of bacteria. They act upon the system to produce the symptoms of the diseases ascribed to the species through whose agency they are manufactured.

Toxalbumins.—These are proteid poisons produced by bacteria, and they give rise to the symptoms of the larger number of infectious diseases.

Leucomaines have been defined as "basic substances which result from tissue metabolism in the body."

Toxins.—This is a general term applied to all poisons produced by bacteria, and especially to those of unknown composition.

Antitoxins.—Bacteria also produce another class of compounds known as antitoxins. These act upon the tissues in such a way as to prevent bacterial infection.

Immunity.—This term is applied to the power of resistance to bacterial infection which may be exerted by an individual man or animal. This may be natural; or it may be acquired by disease, acclimatization, vaccination, the injection of antitoxins, and other means.

An antiseptic is a substance which simply retards the growth of bacteria.

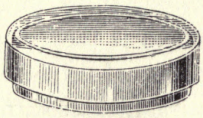
A germicide is a substance which will kill bacteria. The term "disinfectant" has the same significance. Among the commonly used and most effective germicides may be named the following: Carbolic acid, mercuric chloride, silver nitrate, formaldehyde, sulphur dioxid, calcium hypochlorite, lime, potassium permanganate, and copper sulphate.

For the destruction of the sputum of consumptives and the evacuations of cholera and typhoid patients, Crookshank recommends the use of carbolic acid, one in twenty, or a strong solution of chloride of lime. Disinfection of the skin is often difficult, as a number of species are of frequent occurrence upon its surface, such

as *Streptococcus pyogenes, Staphylococcus pyogenes aureus* (also *albus* and *citreus*), *Bacillus tuberculosis, Bacillus pyocyanus,* and others. *Staphylococcus epidermidis albus* finds its normal habitat in the skin. Under ordinary circumstances, sponging the skin with carbolic acid, one in forty, or rinsing it in bichloride of mercury, 1 in 1,000, will give satisfactory results.

For the disinfection of wounds, hydrogen peroxide is recommended. As a wash for the mouth and throat in cases of inflammation, abrasion, and suppuration, a weak solution of permanganate of potash will be found very efficient when used as a gargle.

Petri Dish.



Bausch & Lomb Optical Co.

MEMORANDA.

PART IV.

URINALYSIS.

The clinical significance of urinalysis renders this subject of paramount importance to the physician. Only a bare outline is here given of some of the important processes of physical and chemical urinalysis. In the preparation of this outline Purdy's "Practical Urinalysis and Urinary Diagnosis" has been consulted. Those who may contemplate pursuing these investigations more exhaustively will not be disappointed if they secure this admirable text. The microscopical examination of urine is valuable in demonstrating pus, bacteria, animal parasites, blood, fat, epithelium, inorganic crystals, and other products, thus affording very valuable assistance in the detection of abnormal conditions. The few practical exercises pointed out in this brief discussion are only suggestive of the field which might be explored by the ambitious student.

CHAPTER XXX.

PHYSICAL, CHEMICAL, AND MICROSCOPICAL URINALYSIS.

1. PHYSICAL URINALYSIS.

The physical examination of urine includes the following determinations:

1. The amount voided in twenty-four hours.—This determination is important as a basis upon which to estimate the quantity of solid matter eliminated in a given period. It also indicates the possible presence or absence of such diseases as uræmia, diabetes, and Bright's disease. The normal quantity passed in twenty-four hours ranges from 1,000 c.c. to 2,000 c.c., the average in a healthy person being 1,500 c.c.

2. Odor.—The odor may be aromatic, ammoniacal, putrid, or scarcely perceptible. The odor of freshly-voided normal urine is

slightly aromatic. A putrid odor indicates tissue degeneration or the decomposition of the urine within the body.

3. Color.—The color of normal urine ranges from that of water, through the yellows, to reddish brown, the average being straw, or amber, color. The color is due to certain pigments. Concentrated urine is more highly colored than that of low specific gravity. Abnormal urine exhibits greater fluctuations in color than that of health. Red may indicate the presence of blood; a black color indicates a certain form of cancer; green indicates jaundice, and occurs sometimes in diabetes; blue occurs in cholera and typhus.

4. Transparency.—Normal urine is transparent when voided, becoming cloudy after standing, owing to the action of bacteria. Pathological urine is often cloudy when first obtained, due to the action of bacteria, the presence of blood, pus, etc., and the precipitation of salts. Heat removes cloudiness due to precipitated urates, but not when caused by bacteria, pus, or precipitated phosphates. Acids will clear up any cloudiness due to precipitated phosphates, but will increase turbidity arising from bacteria, albuminous casts, and pus.

5. Chemical reaction.—This test consists in finding the action of urine on litmus, an acid urine turning blue litmus red, and that which is alkaline changing red to blue. Normal urine is acid, the acidity being due to the acid sodium phosphate. Excessive acidity is calculated to irritate the urinary passages and favors the formation of uric acid concretions. Alkalinity of the urine may be due to the presence of ammonium carbonate (resulting from the decomposition of urea by the agency of bacteria) or to an alkali of sodium or potassium. In the former case the litmus paper turns red again upon drying; in the latter case it remains blue upon drying.

6. Specific gravity.—Normal urine (1,500 c.c.) has a specific gravity ranging from 1.015 to 1.025, the average being 1.020, water being taken as the standard. Low specific gravity may indicate nephritis and organic albuminuria, though in functional albuminuria the specific gravity is above normal. High specific gravity is suggestive of melituria, and when it reaches 1.030 there is indicated

the probable presence of sugar. The determination of specific gravity is made with a urinometer.

7. Solids.—Normal urine, when freshly voided, is free from visible solids, save a few epithelial scales. The visible solids of abnormal urine may be estimated by means of the centrifuge. The quantity of invisible solids in normal or pathological urine may be estimated by multiplying the last two figures of the number representing the specific gravity by 2.33. This gives the number of grams in a liter of the sample. Thus, in urine whose specific gravity is 1.030, the number of grams of solids held in solution would be 30×2.33, or 69.9 grams. The amount of solids eliminated by the urine, 1,500 c.c. in twenty-four hours, would be represented by the equation: $20 \times 2.33 \times 1.5 = 69.9$ grams. The quantity of solids for twenty-four hours is affected by age, diet, exercise, etc., the average quantity for a person of 145 pounds being 61 grams. A reduction of solids indicates renal diseases (with a tendency toward uræmia) and defective elimination. The importance of knowing the amount of urine passed in twenty-four hours is evident.

Laboratory exercise No. 66.—Make a physical examination of some sample of urine, performing all the tests indicated above.

2. CHEMICAL URINALYSIS.

1. Urea, $CO(NH_2)_2$.—The normal quantity of urea in the urine is about one-half of all the solid constituents, or about 35 grams. It is formed in the liver as the result of destructive metabolism of the tissues and the splitting up of nitrogenous food principles. An excess of urea occurs in acute diseases, such as fevers, in some liver affections, such as diabetes, and accompanies excessive physical and mental exertion, and indicates tissue waste A deficiency occurs in chronic diseases. The average elimination of urea for an adult for twenty-four hours is estimated at 33 grams. The percentage in the urine is estimated by means of the Doremus ureometer as indicated in the following method: Fill the long arm of the ureometer with hypobromite. By means of the graduated pipette add 1 c.c. of urine by compressing the nipple gently and steadily. The hypobromite causes the liberation of nitrogen gas, which collects in the

upper end of the cylinder. The readings on the ureometer will indicate the number of milligrams of urea in 1 c.c.; from this determination may be calculated the total amount eliminated in twenty-four hours.

Hypobromite may be prepared as follows: To 250 c.c. of water add 100 grams of sodium hydrate. When ready to make the test, add to 10 c.c of the sodium hydrate 1 c.c. of bromine, and then a quantity of water equal to this mixture.

2. Uric acid.—Uric acid is a nitrogenous compound supposed to be formed in the liver by the union of ammonia and lactic acid. The quantity eliminated in twenty-four hours by the healthy adult is about 0.5 gram. An excess occurs in leukæmia, fevers, lung and heart diseases, tumors, etc.; an absence of uric acid occurs in Bright's disease, gout, and other affections.

A qualitative test may be made by strongly acidulating with hydrochloric acid a beakerful of urine. After standing twenty-four hours, uric acid crystals will be deposited, which may be examined with the microscope.

3. Glucose.—Sugar occurs temporarily in the urine with such diseases as cholera, gout, intermittent fever, etc. Its presence becomes persistent in diabetes. It may be detected by Fehling's solution, Haynes' test, fermentation test, etc.

Fehling's solution is prepared by dissolving 6.9 grams of copper sulphate in 100 c.c. of distilled water. Then a second solution is prepared by dissolving 34 grams of potassium sodium tartrate and 25 grams of potassium hydrate in 100 c.c. of water. In making the test, place about 5 c.c. of each of these solutions in separate test tubes, heat to boiling, and, after adding one to the other, add a few drops of the suspected urine. If a yellowish-red precipitate is formed, it indicates the presence of sugar.

Haynes' solution is prepared by mixing 30 grains of copper sulphate with one-half ounce of distilled water, then adding one-half ounce of pure glycerine, and, after mixing, adding five ounces of liquor potassæ The test is made by boiling five to ten cubic centimeters of this solution in a test tube, and adding six to eight drops

of the suspected urine. Boil again, and, if sugar be present, a yellowish-red precipitate will be formed.

A quantitative test for sugar may be made with the fermentation saccharometer as follows: To 10 c.c. of urine add one gram of Fleischmann's yeast; shake thoroughly in a test tube; pour the mixture into the saccharometer. The yeast produces the decomposition of the sugar with the formation of carbonic acid gas. The quantity of gas evolved indicates the amount of sugar present, and may be determined by the readings of the graduated scale.

4. Albumin.—The presence of albumin in urine may be due to degeneration of the kidney tissues, excessive blood pressure, or an increased diffusibility of the serum-albumin of the blood. It is probably more often due to kidney degeneration, and in such cases is indicative of chronic albuminuria, known as Bright's disease. It may be detected by the following methods:

(1) *Heat Test.*—Pour into a test tube about 10 c.c. of the suspected urine; heat the upper portion to boiling. If a cloudiness appears, which is not removed by nitric acid, albumin is present.

(2) *Nitric Acid Test.*—Pour into a test tube 5 c.c. of nitric acid; then, with a pipette, add, drop by drop, some of the suspected urine, allowing it to run down the side of the tube. If albumin is present, a white ring will be formed at the plane of juncture of the two fluids.

(3) *Quantitative Test.*—Using Esbach's albuminometer, fill the tube with urine to the graduation U; then add the test solution (10 grams picric acid, 20 grams citric acid, water to make one liter) to fill the tube to graduation R. Cover the end of the tube and shake the contents thoroughly; close the tube with rubber stopper, and set aside for twenty-four hours. The precipitated albumin can then be estimated from the graduated scale, each graduation indicating one gram of albumin in a liter of urine.

5. Chlorides.—The quantity of chlorides eliminated by the urine in twenty-four hours is from six to ten grams. When the amount becomes less than five grams, it indicates weakness of digestion. An excessive excretion occurs in diabetes, and is considered a favor-

able sign in dropsical conditions. The presence of chlorides may be tested by acidulating with nitric acid and adding silver nitrate. A white precipitate of chlorides is formed.

Quantitative test: Dilute 10 c.c. of urine with 100 c.c. of water; add a few drops of potassium chromate solution; then add slowly a solution of silver nitrate (17 grams to a liter of water) until the color of the solution changes from yellow to red. Each c.c. of silver nitrate (standard solution) used will precipitate 0.00354 gram of chlorine, from which may be estimated the percentage by weight of chlorine in the urine.

6. Phosphates.—The earthy phosphates are those of calcium and magnesium; the alkaline phosphates are those of sodium and potassium; triple phosphate is ammonio-magnesium phosphate; the acid phosphates of the alkalies give the acid reaction to the urine, and are represented by the formulas NaH_2PO_4 and KH_2PO_4. An excess of phosphates occurs in diabetes. A diminution generally occurs in nephritis, gout, rheumatism, and acute infectious diseases.

The earthy phosphates may be detected by adding ammonium hydrate and gently heating; a white precipitate is formed, which is dissolved by the addition of acetic acid. A quantitative determination may be made by filling a test tube whose diameter is two centimeters with urine to the depth of 5.3 centimeters; to this add a few drops of ammonium hydrate and heat until the phosphates are precipitated; set aside and in fifteen minutes examine. If the height of sediment be 1 centimeter, the quantity of earthy phosphates is normal, but diminished or increased if the height should be less or greater than 1 centimeter.

To determine approximately the quantity of alkaline phosphates, proceed as follows: Remove the earthy phosphates by precipitation and filtration, and to 10 c.c. of the filtered urine add 3 c.c. of magnesium mixture. Magnesium fluid is prepared by dissolving magnesium sulphate and ammonium chloride, one part each, in eight parts of distilled water and one part of ammonium hydrate. The amount of turbidity formed by the precipitate indicates the quantity of alkaline phosphates present. If it is simply milky, the quantity is

normal; if heavy, increased; and if no precipitate, there is a decrease.

7. Sulphates.—The total amount of sulphuric acid in combination excreted by an adult in twenty-four hours is between two and three grams. An increase of sulphates occurs in serious stoppages of the food in the intestines, the pus forming diseases, as in fetid bronchitis, diphtheria, etc., and in acute fevers, meningitis, and rheumatism. They may be detected by adding to a portion of the urine one-third the amount of barium chloride acidulated with hydrochloric acid. A white, milky precipitate indicates the presence of sulphates. An approximate quantitative determination may be made as follows: To 10 c.c. of urine add 3 c.c. of barium chloride solution, which is prepared by mixing four parts of barium chloride, one part of hydrochloric acid, and sixteen parts of distilled water. If a milky turbidity results, the quantity of sulphates is normal; if the precipitate is heavy, having the consistency of cream, it is increased.

Laboratory exercise No. 67.—*Chemical examination.* Make an analysis of a sample of urine by the chemical tests suggested above. Write out your analysis in systematic form.

3. MICROSCOPICAL URINALYSIS.

The microscopical examination of urine is of value in confirming the results of physical and chemical analyses and in throwing light upon certain pathological conditions—light obtainable from no other source. The sediments of urine may be organized or unorganized. Organized sediments comprise epithelium, blood, pus, tubular casts, spermatozoa, bacteria, and vermes. The unorganized sediments comprise crystals of the phosphates, urates, etc., amorphous compounds, and inorganic concretions.

UNORGANIZED SEDIMENTS.

Among some of the forms of crystals which may be demonstrated by microscopical urinalysis are those of calcium oxalate (Fig. 1), triple phosphate (Fig. 2), uric acid (Fig. 3), leucin and tyrosin (Fig. 4), nitrate of urea (Fig. 5), calcium sulphate (Fig. 6), calcium phosphate (Fig. 7), and hæmin (Fig. 8).

UNORGANIZED SEDIMENTS.

Fig. 1

Calcium Oxalate.

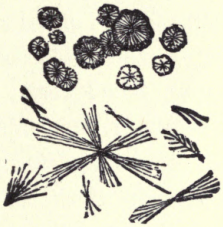
Fig. 2

Triple Phosphate.

Fig. 3

Uric Acid.

Fig. 4

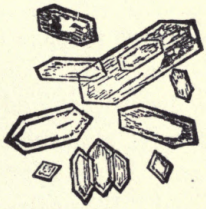


Leucin and Tyrosin.

Fig. 5

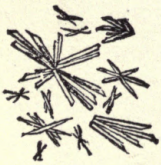
Nitrate of Urea.

Fig. 6



Calcium Sulphate.

Fig. 7



Calcium Phosphate.

Fig. 8

Hæmin Crystals.

Amorphous sediments, such as urates, phosphates, etc., are of occasional occurrence.

Laboratory exercise No. 68.—*Organic sediments.* Obtain some cystitic urine by means of a pipette; apply a drop of the sediment to the slide; cover and search for epithelium, noting the forms of the cells; search also for pus and bacteria. Of what significance are these elements?

Bacillus coli commune, Micrococcus ureæ, and the species of *Staphylococcus* invariably occur in cystitic urine. What other elements do you observe? Make a cover-glass preparation, and stain with gentian-violet. Mount in water, and make a search for pus, bacteria, epithelium, blood, crystals, and other structures.

Obtain samples of urine from cases of nephritis, Bright's disease, etc. Examine the sediments by the usual method, and demonstrate epithelium, blood casts, bacteria, etc., such as may be present. Fatty, granular, epithelial, and blood casts may be easily demonstrated. Hyaline casts should be precipitated by means of a centrifuge. A drop of the sediment is placed upon a slide containing a cell, covered, and then examined. The casts are of small or large diameter and transparent.

Laboratory exercise No. 69.—*Crystals.* Allow cystitic urine to stand for twenty-four hours. Examine some of the sediment and determine the kinds of crystals present by comparing their forms with those of the illustrations on page 179.

Clean five slips; upon each place a drop of urine. Allow the first to dry without adding any reagent; to the second add a small drop of ammonium hydrate; to the third, a drop of hydrochloric acid; to the fourth, nitric acid; and to the fifth, dilute sulphuric acid. When these preparations are dry, or nearly so, cover and examine. Preparation No. 1 may exhibit crystals of calcium oxalate, leucin, tyrosin, and uric acid; No. 2 will exhibit crystals of triple phosphate (Fig. 2) and calcium phosphate (Fig. 7); No. 3 may illustrate crystals of uric acid (Fig. 3); No. 4 will exhibit nitrate of urea (Fig 5); and No. 5 will show crystals of calcium sulphate (Fig. 6). Determine any other forms which may be observed.

ORGANIZED SEDIMENTS.

1. Epithelium.—Epithelium occurs in urine as isolated cells; or, occasionally, groups of attached cells may be demonstrated. In normal urine there is always a limited number of epithelial cells due to ordinary desquamation, but under pathological conditions the number becomes greatly increased. This may be due to inflammation, suppuration, friction, and the action of bacteria. The elements from the renal tubules are generally small round cells, columnar or cuboidal cells; those from the excretory duct are of the tall columnar variety; and those from the pelvis, ureter, and bladder are of the transitional type, exhibiting irregular forms—spindle-shaped, polyhedral, and large round cells, some having more than one nucleus, and some exhibiting pointed processes; cells from the prostatic portion of the urethra are of the squamous type, while those from the free portion are low columnar cells. In the female, squamous epithelium lines the entire urethra.

2. Blood (Fig. 10).—Blood occurs only in pathological urine; it may be detected microscopically by the presence of the red disks. These are known by their form and the absence of nuclei; they seldom form in rouleaux, but often exhibit crenation. Tuberculosis of the kidneys, pyelitis, cystitis, and other affections are suggested by the presence of blood.

3. Pus (Figs. 9 and 14).—Pus consists of dead or dying leucocytes, which have escaped from the vascular channels. Leucocytes are concerned in repairing diseased tissues and the destruction of microbes. Vast numbers die in the conflict, and these constitute pus. Pus cells may be distinguished from other elements by their size, granular appearance, and nuclei. They often contain several nuclei in each cell and occasionally exhibit amœboid movement. Pus is invariably present in cystitis, gonorrhœa, tuberculosis, etc.

4. Casts.—These originate in the renal tubules and comprise several varieties: Blood casts, epithelial casts, granular casts, fatty casts, and hyaline casts.

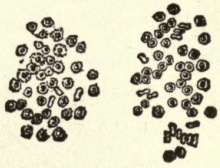
Blood casts (Fig. 13) are the result of hemorrhage in the urinary tubules, and indicate such infections as hæmaturia, renal congestion, and acute nephritis.

ORGANIZED SEDIMENTS.

Fig. 9

Pus Cells.

Fig. 10



Blood.

Fig. 11

Hyaline Casts.

Fig. 12

Epithelial Casts.

Fig. 13

Blood Casts.

Fig. 14

Pus Showing Amœboid Movement.

Fig. 15

Granular Casts.

Epithelial casts (Fig. 12) result from the disintegration of the epithelium of the renal tubules; their presence indicates nephritis and other kidney infections.

Granular casts (Fig. 15) result from the disintegration of pus, epithelium, and blood, and are indicative of pathological conditions of the kidney.

Fatty casts are the result of changes in the kidney which indicate the destruction of the protoplasm of the cells.

Hyaline casts (Fig. 11) are probably produced by the coagulation of certain elements of the blood which have gained access to the renal tubules. They are almost colorless and difficult of demonstration; their presence indicates interstitial-nephritis.

5. Bacteria.—Normal urine is free from bacteria. The species most commonly met with in pathological urine are *Micrococcus ureæ, Staphylococcus pyogenes aureus, albus,* and *citreus, Streptococcus pyogenes* and *Bacillus coli commune. Bacillus tuberculosis* is of occasional occurrence. These organisms can be demonstrated by the usual methods of staining.

6. Vermes.—Two species of vermes are of occasional occurrence —namely, *Distoma hæmatobium* and *Filaria sanguinis hominis.*

Laboratory exercise No. 70.—Make a complete analysis of some sample of urine, and fill out the form presented on pages 184–185.

MEMORANDA.

ANALYSIS OF URINE.

Sample .....

.....

Physical Tests.

1. Amount in 24 hours..
   - Normal urine .....
   - Sample examined .....
2. Odor .....
   - Normal urine .....
   - Sample tested .....
3. Color .....
   - Normal urine .....
   - Sample .....
4. Transparency .....
   - Normal urine .....
   - Sample .....
5. Chemical reaction ...
   - Normal urine .....
   - Sample .....
6. Specific gravity .....
   - Normal urine .....
   - Sample .....
7. Solids .....
   - Visible...
     - Normal .....
     - Sample .....
   - Invisible.
     - Normal .....
     - Sample .....

Chemical Tests.

1. Urea .....
   - Normal .....
   - Sample .....
2. Uric acid .....
   - Normal .....
   - Sample .....
3. Glucose .....
   - Normal .....
   - Sample .....
4. Albumin .....
   - Normal .....
   - Sample .....

5. Chlorides ..... { Normal ..... Sample .....

6. Phosphates ..... { Earthy .. { Normal ..... Sample ..... Alkaline . { Normal ..... Sample .....

7. Sulphates ..... { Normal ..... Sample .....

Microscopical Tests.

1. Epithelium ..... { Small round cells ..... Tall columnar ..... Transitional ..... Squamous ..... Low columnar .....

2. Blood .....

3. Pus .....

4. Casts ..... { Blood casts ..... Epithelial ..... Granular ..... Fatty ..... Hyaline .....

5. Bacteria .....

6. Spermatazoa .....

7. Crystals .....

8. Amorphous sediments .....

Clinical Significance of Examination.

.....

.....

Respectfully,

.....

Date .....19....

INDEX.

MEMORANDA.

MEMORANDA.

MEMORANDA.

MEMORANDA.